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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

October 1, 1977 through September

Part VI

DIVISION OF CANCER TREATMENT

U.S NATIONAL CANCER INSTITUTE
Report of Program Activities
— ANNUAL REPORT

October 1, 1977 through September 30, 1978

TABLE OF CONTENTS

DIVISION OF CANCER TREATMENT

Page

Director - OD

Introduction 1
Publications 6
Code for Division of Cancer Treatment Stratification 9
Table I Analysis of Contract Activities for FY78 11
Table II Analysis of Contracts by Activity for FY78 13
Table III Analysis of Activities by Active Contracts for FY78 43
Table IV Description of Contracts 87

Project Reports

CM-06401-06 Data Processing Systems for Scientific and Management Information 161
CM-07025-04 Biomedical Engineering for Intramural Research 164
CM-09053-03 Physical Science Collaborative Research 168

Section Reports

Assistant Director for International Treatment Research 169
Publications Section 189
Literature Research Section 195

ASSOCIATE DIRECTOR FOR DEVELOPMENTAL THERAPEUTICS PROGRAM

Summary Report 197

Drug Synthesis and Chemistry Branch - DSCB

Summary Report 205
Publications by Contractors 220
Patents by Contractors 225
Publications by Staff 226
Drug Synthesis Section 227
Chemical and Drug Information Section 235

Project Report

CM-07101-03 Computer Methods for Drug Preselection Based on Structure-Activity 244

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1978
Pt. 6

Page

Natural Products Branch - NPB

Summary Report 251
Publications by Contractors 264

Drug Evaluation Branch - DEB

Summary Report 271
Publications by Staff 288
Publications by Contractors 290
Automated Information Section 293
Mammalian Genetics and Animal Production Section 297
Screening Section 301

Pharmaceutical Resources Branch - PRB

Summary Report 323
Publications by Staff 327
Publications by Contractors 328
Patents by Contractors 329
Chemical Resources Section 331
Analytical and Product Development Section 337
Clinical Products Section 343

Project Report

CM-03584-06 Research in the Dosage Form Development of New Antitumor Drugs 348

Laboratory of Medicinal Chemistry and Biology - LMCB

Summary Report 355

Project Reports

CM-06144-01 Tyrosinase Activated Cytotoxic Agents 367
CM-07102-03 Pharmacology of Maytansine, Dimethylbenzyl-rifampicin, and other Ansa Macrolides 369
CM-07103-03 Studies on the Clinical Pharmacology of the Folic Acid Antagonists 372
CM-07105-03 Biochemical and Pharmacological Studies of Pyrimidine Nucleoside Analogs with Antitumor Activity 375
CM-07106-03 Adenosine Deaminase Inhibitors as Potentiators of the Pharmacological Activity of Adenosine Analogs 377
CM-07107-03 Biological Studies on Metastatic and Non-Metastatic Melanomas 379

Laboratory of Medicinal Chemistry and Biology - LMCB (Cont'd.) Page

CM-07108-02 Adenosine Deaminase Inhibitors as Immunosuppressive Agents 381

Applied Pharmacology Section - LMCB

Summary Report 385

Project Reports

CM-06145-01 Characterization of Adenosine Deaminase in Normal and Neoplastic Tissues 386

CM-07109-02 Effect of Anticancer Drugs on the Synthesis and Processing of Nuclear RNA 389

CM-07110-02 Effect of Anticancer Drugs on the Translation of Messenger RNA *in vitro* 394

CM-07111-02 Action of Anticancer Drugs on the Phosphorylation and Activity of Nonhistone Chromosomal Proteins 397

Drug Design and Chemistry Section - LMCB

Summary Report 401

Project Reports

CM-03580-09 Chemical Research in the Development of New Anticancer Drugs 402

CM-03581-09 The Analytical Chemistry of New Anticancer Drugs 409

CM-03582-04 Structure-Activity Studies of Anticancer Drugs 414

Molecular Biology and Methods Development Section - LMCB

Project Reports

CM-03579-05 Effect of Chemotherapeutic Agents on L1210 Cell Cycle Progression 418

CM-07104-03 Nutritional and Pharmacological Regimens for Potentiation of Chemotherapeutic Activities 422

Laboratory of Toxicology - LT

Summary Report 425

Project Reports

CM-03518-10 Pharmacologic Interruption of the Metabolism of the Dicarboxylic Amino Acids and their Amides 435

CM-06110-08 The Pathology of Drug-Induced Alterations in Animals and Man 446

CM-06122-06 Drug Interactions and other Factors Influencing the Physiological Disposition and Toxicity of Drugs 453

CM-06143-01 Selected Transport, Metabolic and Toxicologic Characteristics of Antineoplastic Agents 468

Laboratory of Chemical Pharmacology - LCHP

		Page
Summary Report		475
Project Reports		
CM-03500-14	The Distribution of Drugs Between Blood, Brain and Cerebrospinal Fluid	485
CM-03502-15	Folic Acid Antagonists in Experimental Tumors and Normal Tissues of Mice	494
CM-03505-15	Potential Embryotoxic, Carcinogenic and other Adverse Effects of Anticancer Agents	497
CM-03506-15	Pharmacology and Disposition of Antitumor Drugs	504
CM-03509-15	Carcinogenesis, Chemotherapy and Biological Markers in Non-Human Primates	511
CM-06108-09	Studies on Mechanism of Action and Mechanisms of Resistance of Antitumor Agents	520
CM-06134-03	The Role of the Lymphatic System in the Absorption and Distribution of Antitumor Agents	526
CM-06136-02	Chemically Induced Cell Differentiation in Neoplasia	530
CM-06137-02	Mechanism of Chemically Induced Hepatomas and Ovarian Tumors	533
CM-06138-02	Mutagenicity and Carcinogenicity of Antitumor Agents and Chemical Carcinogens	538
CM-06141-01	Kinetics and Pharmacologic Effects of Polymer Linked Antitumor Agents	544
CM-06142-01	Thymidine Pharmacology in Mice and Man	547
CM-07100-05	Tumor Biology and Chemotherapy	549

Laboratory of Molecular Pharmacology - LMPH

Summary Report		555
Project Reports		
CM-06112-06	Effects of Chemotherapeutic Agents on DNA	559
CM-06132-02	Biological Effects of Chemotherapeutic Agents on Cultured Human and Rodent Cells	568
CM-06140-02	Protein Interactions in Chromosomes	574

Laboratory of Tumor Cell Biology - LTCB

Summary Report		579
Project Report		
CM-06117-06	Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells	581

ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY PROGRAM — Page

Summary Report 593

Project Report - CO

CM-06308-07 Biostatistics and Data Management Section 599

Clinical Pharmacology Branch - CP

Project Reports

CM-06402-08 Tumor Growth Kinetics and Chemotherapy 609
CM-06510-02 Mechanisms of Tissue Toxicity Caused by Adriamycin 617
CM-06511-02 Molecular Pharmacology of Cyclic Nucleotides 619
CM-06512-02 Molecular Toxicology 622
CM-06513-02 Molecular Pharmacology of Antitumor Agents 630

Medicine Branch - MB

Project Reports

CM-03403-13 Clinical Trials and Miscellaneous Clinical Investigations 633
CM-03404-07 Immunologic Aspects of Cancer 640
CM-03420-09 Kinetics of Cellular Proliferation 642
CM-03423-07 *In Vitro* Marrow Granulocyte Culture 644
CM-03424-01 Whole Body Hyperthermia: Clinical Investigations 646
CM-06119-09 Cytogenetic and Hematopoietic Stem Cell 648
CM-06500-05 Clinical Pharmacology of Antineoplastic Agents 651
CM-06700-05 Clinical Program in Breast Carcinoma 655
CM-06702-03 Mechanisms of Hormone Dependence of Human Malignancy 658

NCI-VA Medical Oncology Branch - VAMO

Summary Report 661

Project Reports

CM-03024-09 Clinical Trials and Miscellaneous Clinical Investigations 674
CM-06575-03 Laboratory Investigation of Tumor Cell Biology 709

Pediatric Oncology Branch - PO

Project Reports

CM-06810-14 Study and Treatment of Cancer in Children and Young Adults 733

Pediatric Oncology Branch - PO (Cont'd.) Page

CM-06820-05 Hematological Support of Patients with Cancer or Aplastic Anemia 757

CM-06830-08 Infectious Complications of Malignancy: Prevention, Diagnosis, and Therapy 772

CM-06840-03 Tumor Immunology and Immune Surveillance 783

CM-06850-08 DNA and RNA Tumor Viruses: Molecular Mechanisms of Infection and Carcinogenesis 792

CM-06860-02 Clinical and Molecular Pharmacology 807

CM-06870-02 Regulation of Differentiation in Normal and Leukemic Hematopoietic Cells 811

CM-06880-01 Experimental Approaches to the Treatment of CNS Malignancy 818

Radiation Oncology Branch - RO

Summary Report 825

Project Reports

CM-00650-23 Service Radiation Therapy 827

CM-00684-23 Non-Clinical Service Irradiation 829

CM-00988-13 The Study of Chronic Lymphocytic Leukemia (CLL) 831

CM-00990-13 The Study of Non-Hodgkin's Lymphomas 833

CM-00994-07 The Study of Small Cell Cancer of the Lung 835

CM-00995-03 Radiation Therapy Planning Using Computerized Tomography Interfaced with Computerized Dosimetry Systems 838

CM-00996-02 Immunologic Function in Patients Receiving Total Body Irradiation 840

CM-00997-02 Service Radiation Physics 843

Surgery Branch - SURG

Summary Report 845

Project Reports

CM-03800-08 Surgical Consultations and Collaborative Research Involving Surgical Services at the NIH 852

CM-03801-08 Clinical Studies in Cancer Surgery 856

CM-03807-04 Immunoevaluation of Patients with Cancer 863

CM-03811-04 The Immunotherapy of Animal and Human Sarcomas 866

CM-03812-04 Studies with a Chemically-Induced Rat Colon Cancer Model 868

Surgery Branch - SURG (Cont'd.) Page

CM-03826-04 Biologic Tumor Markers in the Cells and Sera of Patients with Urologic Cancers 870

CM-06652-02 Decreasing Strengths of Cell-Mediated Immune Responses Generated Following Repeated Allo-antigenic Stimulation in Inbred Strains of Mice 872

CM-06653-02 Metabolic Studies in Tumor-Bearing Patients and Animals 873

CM-06654-01 Immune Studies in Malignant Disease 875

ASSOCIATE DIRECTOR FOR BALTIMORE CANCER RESEARCH PROGRAM

Summary Report 877

Clinical Oncology Branch - COB

Summary Report 879

Project Reports

CM-06230-09 Diagnostic Laparotomy and Splenectomy for Staging Hodgkin's Disease 881

CM-06259-06 Extension of the Computerized PROMIS System into the Cancer Treatment Area 883

CM-06263-07 Protocol for Staging and Treatment of Lymphosarcoma and Reticulum Cell Sarcoma 886

CM-06275-09 Cell Transfusion Therapy 888

CM-06276-05 The Chemotherapy of Metastatic Testicular Carcinoma 893

CM-06277-07 Chemotherapy of Adult Acute Leukemia 895

CM-06280-06 Combination Chemotherapy for Advanced Hodgkin's Disease 901

CM-06293-07 A Teaching and Consultation Service in Medical Oncology 903

CM-06295-06 Census: A Remote Access Patient Information Storage and Retrieval System 905

CM-06299-05 Therapy of Patients with Metastatic Sarcoma 908

CM-06913-05 Bronchial Brushing for Pulmonary Infiltrates in Patients with Cancer 912

CM-06915-05 Staging Laparotomy and Combined Modality Therapy in Early Hodgkin's Disease 915

CM-06916-05 Pilot Protocol for Treatment of Patients with Stage III and IV Diffuse Histiocytic Lymphoma 917

CM-06917-05 CVP versus CAVP for Disseminated Non-Hodgkin's Lymphomas 919

Clinical Oncology Branch - COB (Cont'd.) Page

CM-06918-05 Streptozotocin, Bleomycin, CCNU & Adriamycin Treatment of Advanced Hodgkin's Disease 921

CM-06919-04 Initial Drug Therapy for Metastatic Renal Cell Carcinoma 923

CM-06921-05 Adriamycin Therapy for Advanced Mycosis Fungoides 925

CM-06939-03 Methotrexate, Oncovin, L'Asparaginase and Dexamethasone Therapy for Adults with Acute Lymphocytic Leukemia 927

CM-06942-03 Development of the Acoustic Microscope as a Useful Tool in Hematology 929

CM-06944-03 Treatment of Oat Cell Carcinoma of the Lung 931

CM-06947-02 Amphotericin-B and Adriamycin Combination Therapy of Refractory Neoplasia 934

CM-06948-02 Treatment of Chronic Myelocytic Leukemia 936

CM-06949-02 Combination Chemotherapy for Metastatic Carcinoma of the Colon 938

CM-06952-02 Mycosis Fungoides Cooperative Study Group 940

CM-06953-02 Chemotherapy for Metastatic Carcinoma of the Breast 942

CM-06954-02 Chemotherapy for Carcinoma of the Head and Neck 944

CM-06955-02 Adjuvant Chemotherapy and Combination Chemoimmunotherapy in the Treatment of Advanced Malignant Melanoma 946

CM-06956-02 Measurement of 5-Hydroxyindoleacetic Acid and Beta-2 Microglobulin in Cerebrospinal Fluid of Cancer Patients 948

CM-06957-02 Nitrous Ocide in the Treatment of Refractory Adult Leukemia 950

CM-06959-02 The Effect of General Anesthetic Agents on Platelet Aggregation 951

CM-06960-02 New Drug Therapy of Previously Treated Adult Leukemia 954

CM-06970-01 Cis-Dichlorodiammine Platinum II Neurotoxicity 956

CM-06971-01 Phase II Study of Dianhydrogalactitol and Piperazinedione in Malignant Brain Tumors 958

CM-06973-01 Phase I Studies of New Drugs 960

CM-06974-01 Whole Body Hyperthermia 962

CM-06975-01 Treatment of Non-Oat-Cell Carcinoma of the Lung 964

CM-06976-01 Augmentation of Alkylating Agents with DMSO 966

Clinical Oncology Branch - COB (Cont'd.) Page

Section of Infection Research

Project Reports

CM-06217-09 Prevention of Infections in Patients with Cancer 969

CM-06264-09 Evaluation of the Types and Causes of Infectious Diseases in Cancer Patients 973

CM-06266-09 Therapy of Suspected Bacterial Infection in Granulocytopenic Patients with Cancer 977

CM-06950-02 Therapy of Fungal Infections in Patients with Cancer 982

CM-06951-02 Infection Research in Oncology 984

CM-06988-01 Radiation-Related Thyroid Dysfunction in Hodgkin's Disease 986

Research Microbiology Section - COB

Project Reports

CM-06027-09 Microbiological Services for Clinical and Research Studies of Cancer Patients 989

CM-06039-09 Computerized Microbiology Reporting and Data Retrieval Systems 991

CM-06219-09 Studies on Cancer Patient Microbial Flora: Acquisition, Colonization, and Infection 993

CM-06223-09 Studies on Pseudomonas aeruginosa in a Cancer Research Center 996

CM-06254-09 Research Activities Directed Toward Improvement of Microbiology Laboratory Techniques 998

CM-06938-04 Environmental Studies to Prevent Hospital Acquisition of Potentially Dangerous Microflora 1001

CM-06972-01 Antibiotic Studies: Evaluation of New Drugs and Their Characteristics 1004

Clinical Research Pharmacy Section - COB

Project Reports

CM-06043-07 Automated Monitoring of Drug Therapy 1007

CM-06236-07 Role of the Pharmacist in Obtaining a Patient's Drug and Allergy History 1009

CM-06237-07 Development of a Clinical Research Pharmacy Service 1011

CM-06912-04 Antineoplastic Drug Displays for the Computerized Problem-Oriented Medical Information System 1014

Clinical Oncology Branch - COP (Cont'd.)

Page

Clinical Research Pharmacy Section - COP (Cont'd.)

CM-06937-03 A Patient Oriented Outpatient Drug Distribution Service 1016

CM-06238-07 Development of a Unit Dose Service and the Preparation of Parenteral Drugs 1018

CM-06943-03 Audit of the Outpatient Drug Profile 1020

CM-06961-02 Establishment of a Pharmacy Clinical Clerkship in Oncology 1022

CM-06990-01 The Role of the Pharmacist in Monitoring Patients Receiving Ototoxic Drugs 1024

CM-06991-01 Audit of the deHaen Drugs-in-Use Drug Information System 1026

Laboratory of Clinical Biochemistry - LCB

Summary Report 1029

Project Reports

CM-06012-08 Biochemical Pharmacology of Antineoplastic and Other Agents 1031

CM-06014-08 Cellular Control Mechanisms Affecting Cell Growth and Differentiation 1034

CM-06016-08 Pharmacodynamics of Cancer Chemotherapeutic Agents 1037

CM-06241-08 Clinical Pharmacology of Antineoplastic and Other Drugs 1040

CM-06969-01 Laser Cytometry in Quantitation of Lymphocyte Transformation 1043

CM-06992-01 Cell Mediated Immune Response in AKR Lymphoma 1045

Laboratory of Molecular Biology - LMB

Summary Report 1047

Project Reports

CM-06242-08 Regulation of Poly(A) Induced RNase Inhibition by Metals or Polyamines 1049

CM-06933-03 Ribonuclease Activities in Human Sera 1052

CM-06965-02 Analysis of DNA Damage *in vivo* by Alkylating Agents and Ionizing Radiation 1054

CM-06977-01 NMR Study of the Interactions of RNases and Polynucleotides 1056

CM-06978-01 Stability of 5S RNA with Poly(A) Tale and 5S RNA with Poly(A) in *Xenopus Laevis* 1058

Clinical Oncology Branch - COB (Cont'd.) Page

Laboratory of Molecular Biology - LMB (Cont'd.)

CM-06979-01 Isolation and Purification of mRNA from Bovine Pancreas 1060

CM-06980-01 Study of the Physiological Importance of Reverse Transcriptase-P30 Interaction 1062

CM-06981-01 Search for a Compound Which Will Restrict the Binding Between Reverse Transcriptase P30 Complex 1064

CM-06982-01 Study of the Binding Between Reverse Transcriptase and Hodgkin's Disease Patient' RNase 1066

CM-06983-01 Inhibition with Poly(A)-Poly(U) of Endogenous C-Type Virus Expression in the AKR Mouse 1068

CM-06984-01 Study of Reverse Transcriptase-P30 Interaction in Relation to DNA Synthesis 1070

CM-06985-01 Polypeptides Involved in Herpes Simplex Virus Replication and Cell Transformation 1072

CM-06986-01 Control Mechanisms by RNase C Type Virus Replication in the Balb/C Mouse 1074

CM-06987-01 Mapping of the Promotor Regions and the Polypeptide Products of HSV-1 and HSV-2 1076

CM-06989-01 Functional Significance of Polyadenylic Acid 1078

ASSOCIATE DIRECTOR FOR CANCER THERAPY EVALUATION PROGRAM

Summary Report 1081

Clinical Investigations Branch - CIB

Summary Report 1111

Investigational Drug Branch - ID

Summary Report 1133

Project Report

CM-03579-05 Effect of Chemotherapeutic Agents on L1210 Cell Cycle Progression 1148

ANNUAL REPORT

DIVISION OF CANCER TREATMENT

Vincent T. DeVita, Jr., M.D.

Director

The Division of Cancer Treatment is responsible for the development and evaluation of new and improved treatments for the control and cure of cancer, utilizing all modes of therapy. It is the primary component of the National Cancer Program responsible for coordinating cancer treatment within the various elements of the National Cancer Institute, the country and the world.

During the past year, the NCI has carried out a major reorganization, combining within a single organization related activities (e.g., treatment) funded by either contract or grant as well as intramural programs, while separating review functions from operating programs. Thus, the DCT has assumed responsibility for traditional research grants (R01) and program project grants (P01) in the areas of Drug Development and Pharmacology and in Clinical Oncology and Radiation Development, incorporating them into the overall divisional activities in Preclinical and Clinical Treatment Research, respectively. In order to carry out these combined activities more effectively, the Division has itself been reorganized, as shown in Figure I. The major changes are in the Developmental Therapeutics and the Cancer Therapy Evaluation Programs that will be responsible for preclinical and clinical grants. Thus, in Developmental Therapeutics, an Office of Extramural Research and Resources and an Experimental Therapeutics Branch have been established. In the Cancer Therapy Evaluation Program, a Development Branch and a Clinical Projects Branch have been established, in addition to which a Surgery and Nutrition Section has been established in the Clinical Investigations Branch to coordinate programs in those areas.

The reorganization described above was approved and implemented toward the end of the fiscal year. For that reason, the activities covered in this Report, as well as those of the individual Associate Directors and their respective Laboratory and Branch Chiefs, reflect those programs for which the Division was responsible prior to the reorganization. Although those Reports will present the summaries of work carried out in the various components of the program, a few selected highlights will be presented here, as well as a brief summary of some of the activities operated out of the Office of the Director.

The Developmental Therapeutics Program, under Dr. Vincent T. Oliverio as Associate Director, is responsible for essentially all preclinical aspects of the program, including drug design and acquisition; development and use of experimental test systems for drug evaluation; detailed experimental evaluation of drugs individually and in combination with other drugs and treatment modalities; production and formulation of drugs for preclinical and clinical evaluation; and studies on the preclinical toxicology, pharmacology and biochemistry of drugs of interest.

DCT
APPROVED REORGANIZATION STRUCTURE

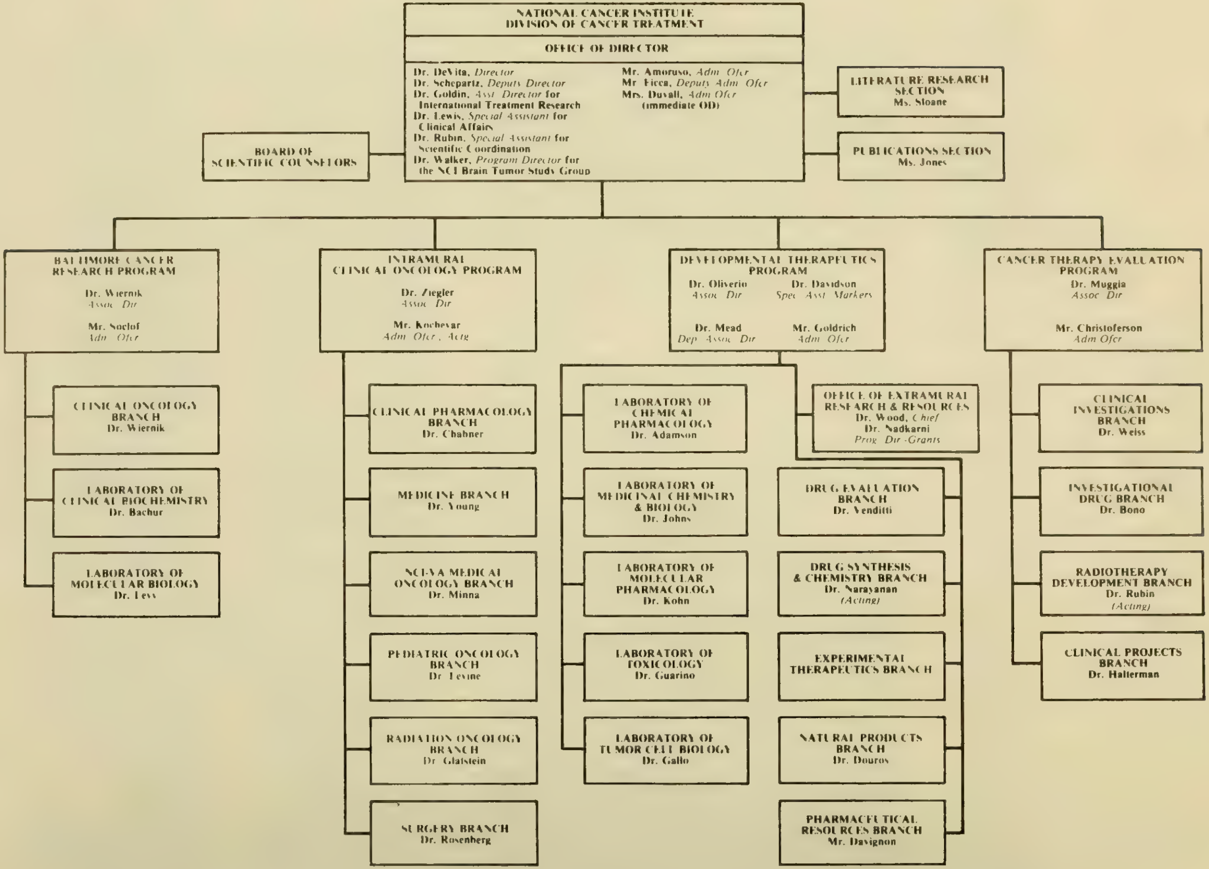
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FIGURE I

During the past year, a group of high priority compounds were identified in preclinical development. One of these, PALA, was selected for rapid movement to clinical trials, and it was found that preclinical toxicology studies could be completed in about half the normal time, albeit at about double the cost. It demonstrated the feasibility of such an approach, however, if circumstances justify the expense.

As described in last year's Report, the antitumor screen has undergone a major change. In spite of a substantial decrease in input, now at a level of about 15,000 synthetic compounds annually, the output of active materials has been maintained.

The Cancer Therapy Evaluation Program, headed by Dr. Franco Muggia as Associate Director, is responsible for almost all extramural clinical activities of the DCT. In preparing for clinical trials, they assemble the preclinical information required by the Food and Drug Administration and serve as liaison with that organization. They are responsible for the initiation and monitoring of all clinical trials conducted under the auspices of the Division. These include Phase I and Phase II clinical trials and in some instances disease-specific Phase III clinical trials under contract. In addition, the grant-supported Clinical Cooperative Group Program is in this area. In carrying out these responsibilities, the Cancer Therapy Evaluation Program has divided the clinical drugs into three categories for purposes of drug distribution. Group A drugs are those in Phase I and early Phase II trials. Distribution of those drugs is limited to contract-supported investigators and others who are members of the Phase I Working Group. Once a drug has reached broader Phase II trials, it enters Group B and is then made available to Cooperative Groups and other investigators through Cancer Center Directors. Once a drug has demonstrated activity in one or more tumor types, it is then moved to Group C (with the approval of the FDA). Drugs in that category can be distributed to investigators with only limited reporting requirements. In that way, drugs that will probably become commercially available but which are not yet in that category can be provided to investigators for the treatment of selected patients without excessive administrative burden.

During 1977, seven new Investigational New Drug Applications (IND's) were submitted to the FDA and clinical studies initiated. In addition, one drug, L-asparaginase, became commercially available in April 1978. Of particular interest during the past year has been the demonstrated activity of cis-platinum diamminedichloride in testicular cancer and head and neck cancer in addition to the prior demonstrated effectiveness in ovarian cancer. It is likely that this compound will become commercially available this year. Other clinical highlights are summarized in the Report of the Associate Director.

Two other programs within DCT are primarily concerning with conducting intramural laboratory and clinical research on specific problems of interest to investigators at NCI.

The Clinical Oncology Program, headed by Dr. John Ziegler as Associate Director, who also serves as Deputy Clinical Director, consists of six intramural Branches--Surgery, Radiation Oncology, Pediatric Oncology, Medicine and Clinical

Pharmacology in Bethesda and a Medical Oncology Branch located at the Veterans Administration Hospital in Washington, D.C. Each of these Branches identifies specific disease areas to focus their attention on. In conjunction with therapeutic trials in these specific areas, investigators in the laboratory explore basic biochemical mechanisms, pharmacological problems, and other fundamental problems of direct concern to the clinical situations. The Clinical Pharmacology Branch is primarily concerned with problems of that nature and collaborates closely with the other Branches. The Report of the Associate Director contains a very concise summary of highlights from each of the Branches.

The Baltimore Cancer Research Program, under Dr. Peter Wiernik as Associate Director, consists of a Clinical Branch located at the University of Maryland Hospital, and Laboratories of Clinical Biochemistry and Molecular Biology at the Baltimore Public Health Service Hospital. The difficulties inherent in this physical separation will be solved this year with the relocation of the laboratories to the University. The Clinical Branch is concerned with a variety of Phase I, II and III studies including studies with hyperthermia. During the past year, a closer collaboration with investigators in Bethesda has resulted in joint lymphoma and leukemia protocols and joint Phase I studies.

Activities Coordinated Within the Office of the Director

International Treatment Research

The DCT activities in International Treatment Research are coordinated in the Office of the Director by Dr. Abraham Goldin, Assistant Director for International Treatment Research. These activities include liaison offices at the Institut Jules Bordet in Brussels and the Japanese Foundation for Cancer Research in Tokyo; active international working agreements with the USSR, France, Japan and the Pan American Health Organization (PAHO); formal relationships with other countries such as Hungary, Poland, Federal Republic of Germany and Egypt; and close working relationships with leading investigators throughout the world. These programs include exchanges of scientists, drugs, clinical protocols and other expertise, and have resulted in the provision of drugs not otherwise available, valuable exchanges of information and a general enhancement of preclinical and clinical research in this country and abroad. A detailed description of these activities is contained in Dr. Goldin's Report.

Radiation Oncology Program

During the past year, the Radiation Oncology Coordination Subcommittee (ROCS) of the DCT Board of Scientific Counselors and its Working Groups have engaged in the preparation of scientific research plans for the development and expansion of the Radiation Oncology Program. This activity reflects the Division's continuing effort to achieve total program balance in treatment research. In addition, the ROCS and its working groups have made important contributions both in the area of scientific information exchange and collaboration, such as the Workshop on Neurotoxicity of Radiosensitizers and by providing major input to the Division for expanding its research effort in radiation oncology. The overall research plan for radiation oncology that encompasses the areas of radiosensitizers/radioprotectors, hyperthermia, toxicology, immunology, and experimental combined modalities research is in its final stages of preparation for submission to the DCT Board of Scientific Counselors.

As a result of important progress made by the Experimental Combined Modality Study Group concerned with preclinical research on the nature and mechanism of interaction of chemotherapy and radiotherapy, this high priority area of research will be expanded through readvertisement and extension of the original Cancer Research Emphasis Grant (CREG), DCT-3.

In the area of radiosensitizer research, major progress has been made during the past year in the following areas of this relatively new initiative in the treatment research program: (1) specialized analog synthesis contracts have been awarded for the development of new, more effective hypoxic cell radiosensitizer compounds, (2) Phase I clinical trials of misonidazole have been completed by the Radiation Therapy Oncology Group, (3) protocols for Phase II clinical trials of this agent are now in the process of development and implementation by both the Radiation Therapy Oncology Group and the Brain Tumor Study Group, as well as in collaboration with clinical investigators in Canada and Europe, and (4) plans are underway for the implementation of a research program concerned with development and clinical investigation of radioprotective compounds.

Research on Schedule I Drugs

The DCT has been analyzing the role of heroin and tetrahydrocannabinol in the symptomatic support of cancer patients.

Heroin - A number of studies from Great Britain have suggested that heroin is qualitatively as well as quantitatively superior to other opiate analgesics such as morphine in the symptomatic support of patients with advanced cancer. A careful reading of these reports as well as recent statements by the major contributor to this literature, Dr. R. G. Twycross, lead to the conclusion that heroin may be superior to morphine only in its increased solubility, a property which would permit the parenteral administration of a given analgesic dose in a smaller volume of liquid. The National Institute on Drug Abuse has awarded a grant to Memorial Sloan-Kettering Institute (Dr. Raymond Houde) for an in-depth study of opiate analgesics, including heroin. In addition to this research effort, the DCT is sponsoring its own IND for heroin and will support studies of this drug's efficacy in comparison with morphine through clinical investigators currently sponsored by the DCT.

Tetrahydrocannabinol (THC) - Since 1975, there have been reports in the literature that THC has activity against cancer chemotherapy-induced vomiting. In addition, Eli Lilly has been investigating the properties of nabilone, a synthetic analog of THC. In order to determine whether additional work is needed in this area, the DCT sponsored a workshop on May 9, 1978, to define the current state of the art of research using these compounds for emesis control. If it is subsequently determined that further studies and research support are needed, then the DCT will obtain an IND for the purpose of sponsoring additional research through its extramural clinical drug development program.

Publications:

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Division of Cancer Treatment Stratification

Code	Description
PDD	Preclinical Drug Development Program -
1XX	Stage I - Acquisition of Materials
2A1	Stage II - Basic Screen - Determination of anti-tumor activity of new agents.
2B1	- Develop acceptable experimental formulation.
2B2	- Verification Screen - Detailed evaluation of new agents -- dose, route, and schedule dependency.
2B3	- Procurement of sufficient amounts of new agent for preclinical studies.
3X1	Stage III - Toxicology and pharmacology in large animals.
3X2	- Production and formulation for clinical trials.
PBR	Preclinical Basic Research -
PBS	Biological Studies - Includes biochemistry, blood products, cell biology, cell kinetics, nutrition, immunobiology, markers, molecular biology, radiobiology, transfusion research, data processing.
PTS	Treatment Studies - Independent treatments, combined modality therapy.
CTR	Clinical Trials Research -
CT1	Phase I Clinical Trials* - initial clinical evaluation of new drugs, clinical pharmacology.
CT2	Phase II Clinical Trials* - allocation for specific disease-oriented resources to study whatever chemotherapy (single agents or combinations) or combined modality regimens have highest priority for initial efficacy evaluation.
CT3	Phase III Clinical Trials* - allocation for specific disease-oriented resources to study whatever chemotherapy or combined modality regimens have highest priority for efficacy evaluation in a controlled clinical setting.

CT4	Phase IV Clinical Trials* - allocation for specific disease-oriented resources to evaluate the combined modality approach to the initial therapeutic attack on local or regional disease in an attempt to increase the number of patients with a long disease-free period.
CT5	Statistics, data processing, other clinical trials research.
CSR	Clinical Trials Supportive Research - Includes Special Pharm./Tox., cell kinetics, markers, blood products, transfusion research, protected environment, hyperthemia, nutrition, statistics, data processing.
MGT	Program Management - Includes administration, dissemination of information to medical and scientific community.

*Supportive care used as ancillary therapy should be prorated among the phases of clinical trials using such resources.

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES FOR FY78

	ANNUAL LEVEL *	PERCENT
PDD PRECLINICAL DRUG DEVELOPMENT PROGRAM	40,705,292	58.91
STAGE I	11,244,390	16.27
1XX ACQUISITION OF MATERIALS		
STAGE IIA	11,130,576	16.11
2A1 DETERMINATION OF ANTI-TUMOR ACTIVITY		
STAGE IIB	8,834,813	12.79
2B1 EXPERIMENTAL FORMULATION DEVELOPMENT	293,376	0.42
2B2 DETAILED EVALUATION OF NEW AGENTS	5,606,004	8.11
2B3 PROCURE. OF AGENTS FOR PRECLIN. STUDIES	2,935,433	4.25
STAGE III	9,495,513	13.74
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	5,219,014	7.55
3X2 PROD. & FORM. FOR CLINICAL TRIALS	4,276,499	6.19
PBR PRECLINICAL BASIC RESEARCH	4,972,794	7.20
PBS BIOLOGICAL STUDIES	2,737,929	3.96
PTS TREATMENT STUDIES	2,234,865	3.23
CTR CLINICAL TRIALS RESEARCH	17,586,473	25.45
CT1 PHASE I CLINICAL TRIALS	851,479	1.23
CT2 PHASE II CLINICAL TRIALS	3,567,205	5.16
CT3 PHASE III CLINICAL TRIALS	6,163,259	8.92
CT4 PHASE IV CLINICAL TRIALS	6,074,376	8.79
CT5 OTHER CLINICAL TRIALS RESEARCH	930,154	1.35
CSR CLINICAL TRIALS SUPPORTIVE RESEARCH	4,485,518	6.49
MGT PROGRAM MANAGEMENT	1,343,825	1.94
TOTAL	69,093,902	100.00

* FY-78 FUNDING REQUIREMENTS AS OF MAY 31, 1978 FOR APPROXIMATELY 281 CONTRACTS. INCLUDED ARE APPROXIMATELY 9 SCHEDULE A CONTRACTS ($692,695) AND 15 SCHEDULE B CONTRACTS ($3,300,000). NOT INCLUDED ARE FUNDS UTILIZED FOR DIRECT PURCHASE OF CLINICAL DRUGS (APPROXIMATELY $2,284,422).

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	AGRICULTURE, DEPARTMENT OF	Y01CM40001	360,000
A.P.	ALABAMA, UNIVERSITY OF	N01CM67083	2,010
O.D.	ARMY, DEPARTMENT OF, FT. DETRICK	Y01C060603	190,858
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53813	214,000
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53846	50,367
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM57006	18,506
D.T.P.	ASSOCIATED BIOMEDIC SYSTEMS, INC.	N01CM02271	200,044
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM57005	100,971
A.P.	BELLAIRE ACRES	N01CM50583	45,864
D.T.P.	BRISTOL-MYERS COMPANY	N01CM77138	260,000
D.T.P.	BRISTOL-MYERS COMPANY	N01CM77147	283,050
D.T.P.	BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223	87,988
A.P.	CHARLES RIVER BREEDING LABS.	N01CM50598	52,104
A.P.	CHARLES RIVER BREEDING LABS.	N01CM53848	46,010
A.P.	CHARLES RIVER BREEDING LABS.	N01CM77141	312,076
D.T.P.	CHEMICAL ABSTRACTS SERVICE	N01CM43722	449,600
D.T.P.	CHESTER BEATTY RESEARCH INSTITUTE	N01CM43736	52,000
D.T.P.	COLLABORATIVE RESEARCH, INC.	N01CM77071	34,844
A.P.	FLOW LABORATORIES, INC.	N01CM50571	45,864
D.T.P.	GRACE, W.R.	N01CM67074	200,000
A.P.	HARLAN INDUSTRIES	N01CM50591	47,424
A.P.	HARLAN INDUSTRIES	N01CM77168	61,838
D.T.P.	HAWAII, UNIVERSITY OF	N01CM33747	86,360
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM33708	78,954
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM57007	82,195

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
A.P.	HEALTH RESEARCH, INC.	N01CM77101	18,053
D.T.P.	HOUSTON, UNIVERSITY OF	N01CM87209	128,101
D.T.P.	IIT RESEARCH INSTITUTE	N01CM43755	60,430
D.T.P.	IIT RESEARCH INSTITUTE	N01CM57004	114,615
D.T.P.	IIT RESEARCH INSTITUTE	N01CM63832	4,108
D.T.P.	ILLINOIS, UNIVERSITY OF	N01CM67090	102,558
O.D.	INSTITUT JULES BORDET	N01CM53840	24,570
D.T.P.	INSTITUTE OF CANCER RESEARCH	N01CM77139	189,000
D.T.P.	IOWA, UNIVERSITY OF	N01CM77176	100,000
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	4,900
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM50577	48,048
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM53775	29,348
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM77165	105,038
A.P.	LITTON BIONETICS, INC.	N01CM53778	14,700
D.T.P.	LITTON BIONETICS, INC.	N01CO75380	1,854,295
D.T.P.	MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM33720	45,665
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57003	33,901
D.T.P.	MIAMI, UNIVERSITY OF	N01CM67077	6,875
D.T.P.	MICROBIAL CHEMISTRY RESEARCH FDN.	N01CM57009	90,000
D.T.P.	MICROBIOLOGICAL ASSOCIATES	N01CM43793	255,000
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM53818	14,000
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM67069	40,030
A.P.	MISSOURI, UNIVERSITY OF	N01CM87157	15,203
A.P.	MURPHY BREEDING LABS., INC.	N01CM50579	44,928
D.T.P.	PARKE, DAVIS AND COMPANY	N01CM77146	565,250

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY	FOR FISCAL YEAR 1978 AS OF 05/31/78		
AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	PFIZER, INC.	NO1CM53805	289,000
D.T.P.	POLYSCIENCES, INC.	NO1CM77070	283,064
D.T.P.	POMONA COLLEGE	NO1CM67062	52,678
D.T.P.	PURDUE UNIVERSITY	NO1CM67091	106,907
D.T.P.	RESEARCH TRIANGLE INSTITUTE	NO1CM67089	164,909
D.T.P.	SAINT JUDE CHILDREN'S RESEARCH HOSPITAL	NO1CM77127	94,000
A.P.	SIMONSEN LABORATORIES	NO1CM50578	48,048
A.P.	SIMONSEN LABORATORIES	NO1CM53847	50,001
A.P.	SIMONSEN LABORATORIES	NO1CM77166	2,267
D.T.P.	SMALL BUSINESS ADMINISTRATION	NO1CM43719	111,927
A.P.	SOUTHERN ANIMAL FARMS	NO1CM50599	45,864
A.P.	SOUTHERN ANIMAL FARMS	NO1CM53845	29,526
D.T.P.	SOUTHERN CALIFORNIA, UNIVERSITY OF	NO1CM67084	138,757
D.T.P.	SOUTHERN RESEARCH INSTITUTE	NO1CM43756	23,000
D.T.P.	SOUTHERN RESEARCH INSTITUTE	NO1CM43762	4,400
D.T.P.	SOUTHWEST FOUNDATION FOR RESEARCH & EDUCATION	NO1CM67075	5,308
A.P.	SPARTAN RESEARCH ANIMALS, INC.	NO1CM50582	22,776
D.T.P.	STANFORD RESEARCH INSTITUTE	NO1CM87207	194,000
D.T.P.	STARKS ASSOCIATES, INC.	NO1CM53745	212,288
D.T.P.	STARKS ASSOCIATES, INC.	NO1CM87206	187,421
A.P.	TACONIC FARMS	NO1CM50597	36,920
D.T.P.	UPJOHN COMPANY	NO1CM77100	818,865
D.T.P.	UTAH, UNIVERSITY OF	NO1CM77142	195,000
D.T.P.	VALUE ENGINEERING COMPANY	NO1CM67086	127,189
D.T.P.	VETERANS ADMINISTRATION HOSPITAL	YO1CM60102	33,318

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	WARF INSTITUTE, INC.	N01CM53770	211,451
D.T.P.	WISCONSIN, UNIVERSITY OF	N01CM67076	2,981
	TOTAL		10,813,408

ANALYSIS OF CONTRACTS BY ACTIVITY

FOR FISCAL YEAR 1978
AS OF 05/31/78

ACTIVITY			
AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
A.P.	ALABAMA, UNIVERSITY OF	N01CM67083	5,526
O.D.	ARMY, DEPARTMENT OF, FT. DETRICK	Y01C060603	47,715
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53813	588,500
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53846	138,510
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM53765	412,500
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM53849	26,602
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM57006	444,155
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM57005	370,228
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM67099	472,500
A.P.	BELLAIRE ACRES	N01CM50583	126,126
D.T.P.	BRISTOL-MYERS COMPANY	N01CM77147	49,950
D.T.P.	CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM67081	181,566
A.P.	CHARLES RIVER BREEDING LABS.	N01CM50598	143,286
A.P.	CHARLES RIVER BREEDING LABS.	N01CM53848	126,528
A.P.	CHARLES RIVER BREEDING LABS.	N01CM77141	858,210
A.P.	FLOW LABORATORIES, INC.	N01CM50571	126,126
A.P.	HARLAN INDUSTRIES	N01CM50591	130,416
A.P.	HARLAN INDUSTRIES	N01CM67079	172,420
A.P.	HARLAN INDUSTRIES	N01CM77168	170,055
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM57007	378,097
A.P.	HEALTH RESEARCH, INC.	N01CM77101	49,647
D.T.P.	IIT RESEARCH INSTITUTE	N01CM43755	196,398
D.T.P.	IIT RESEARCH INSTITUTE	N01CM57004	542,511
D.T.P.	IIT RESEARCH INSTITUTE	N01CM63832	78,043
D.T.P.	INSTITUT JULES BORDET	N01CM57040	143,088

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	19,600
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM50577	132,132
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM53775	80,706
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM77165	288,854
A.P.	LITTON BIONETICS, INC.	N01CM53778	40,425
D.T.P.	LITTON BIONETICS, INC.	N01CO75380	463,574
D.T.P.	MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM33720	213,101
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57003	413,594
A.P.	MASON RESEARCH INSTITUTE	N01CM87164	10,034
D.T.P.	MIAMI, UNIVERSITY OF	N01CM67077	130,633
D.T.P.	MICHIGAN DEPARTMENT OF PUBLIC HEALTH	N01CM33702	64,000
D.T.P.	MICROBIAL CHEMISTRY RESEARCH FDN.	N01CM57009	60,000
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM53818	38,500
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM67069	110,083
A.P.	MISSOURI, UNIVERSITY OF	N01CM87157	41,808
A.P.	MURPHY BREEDING LABS., INC.	N01CM50579	123,552
D.T.P.	PARKE, DAVIS AND COMPANY	N01CM77146	99,750
A.P.	SIMONSEN LABORATORIES	N01CM50578	132,132
A.P.	SIMONSEN LABORATORIES	N01CM53847	137,502
A.P.	SIMONSEN LABORATORIES	N01CM77166	6,235
A.P.	SOUTHERN ANIMAL FARMS	N01CM50599	126,126
A.P.	SOUTHERN ANIMAL FARMS	N01CM53845	81,197
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	736,000
D.T.P.	SOUTHWEST FOUNDATION FOR RESEARCH & EDUCATION	N01CM67075	100,845
A.P.	SPARTAN RESEARCH ANIMALS, INC.	N01CM50582	62,634

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
D.T.P.	STEHLIN FOUNDATION FOR CANCER RESEARCH	N01CM67073	344,390
A.P.	TACONIC FARMS	N01CM50597	101,530
D.T.P.	UPJOHN COMPANY	N01CM77100	144,506
D.T.P.	VALUE ENGINEERING COMPANY	N01CM67086	526,927
D.T.P.	WARF INSTITUTE, INC.	N01CM53770	169,160
D.T.P.	WISCONSIN, UNIVERSITY OF	N01CM67076	56,643
	TOTAL		11,034,876

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II FORMULATION:		
D.T.P.	BEN VENUE LABORATORIES, INC.	N01CM67109	114,995
D.T.P.	HEALTH RESEARCH, INC.	N01CM77118	32,800
D.T.P.	IOWA, UNIVERSITY OF	N01CM43743	48,206
D.T.P.	KANSAS, UNIVERSITY OF	N01CM23217	62,265
D.T.P.	MEDICAL RESEARCH COUNCIL	N01CM87171	8,191
D.T.P.	PHILIPS ROXANE LABORATORIES, INC.	N01CM67053	26,919
	TOTAL		293,376

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
A.P.	ALABAMA, UNIVERSITY OF	N01CM67083	2,512
C.D.	ARMY, DEPARTMENT OF, FT. DETRICK	Y01C060603	119,286
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53813	267,500
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53846	62,959
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM57006	154,221
CTEP	ARTHUR D. LITTLE, INC.	N01CM67047	129,771
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM57005	168,286
A.P.	BELLAIRE ACRES	N01CM50583	57,330
D.T.P.	CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM67081	181,566
A.P.	CHARLES RIVER BREEDING LABS.	N01CM50598	65,130
A.P.	CHARLES RIVER BREEDING LABS.	N01CM53848	57,513
A.P.	CHARLES RIVER BREEDING LABS.	N01CM77141	390,096
D.T.P.	CHESTER BEATTY RESEARCH INSTITUTE	N01CM43736	52,000
D.T.P.	ENERGY RES & DEV ADM	Y01CM10056	30,000
A.P.	FLOW LABORATORIES, INC.	N01CM50571	57,330
A.P.	HARLAN INDUSTRIES	N01CM50591	59,280
A.P.	HARLAN INDUSTRIES	N01CM77168	77,298
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM33708	39,477
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM57007	71,236
A.P.	HEALTH RESEARCH, INC.	N01CM77101	22,567
D.T.P.	HEALTH RESEARCH, INC.	N01CM77118	49,200
D.T.P.	IIT RESEARCH INSTITUTE	N01CM43755	45,323
D.T.P.	IIT RESEARCH INSTITUTE	N01CM57004	84,051
D.T.P.	INSTITUT JULES BORDET	N01CM57040	35,772
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM50577	60,060

ANALYSIS OF CONTRACTS BY ACTIVITY

FOR FISCAL YEAR 1978
AS OF 05/31/78

ACTIVITY AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM53775	36,685
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM77165	131,297
A.P.	LITTON BIONETICS, INC.	N01CM53778	18,375
D.T.P.	LITTON BIONETICS, INC.	N01CO75380	1,158,934
D.T.P.	MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM33720	45,665
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57003	162,726
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57030	173,700
D.T.P.	MEDICAL RESEARCH COUNCIL	N01CM87171	12,286
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM53818	17,500
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM67069	50,038
A.P.	MISSOURI, UNIVERSITY OF	N01CM87157	19,004
A.P.	MURPHY BREEDING LABS., INC.	N01CM50579	56,160
A.P.	SIMONSEN LABORATORIES	N01CM50578	60,060
A.P.	SIMONSEN LABORATORIES	N01CM53847	62,501
A.P.	SIMONSEN LABORATORIES	N01CM77166	2,834
A.P.	SOUTHERN ANIMAL FARMS	N01CM50599	57,330
A.P.	SOUTHERN ANIMAL FARMS	N01CM53845	36,908
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	598,000
A.P.	SPARTAN RESEARCH ANIMALS, INC.	N01CM50582	28,470
A.P.	TACONIC FARMS	N01CM50597	46,150
D.T.P.	TEXAS, UNIVERSITY OF	N01CM53767	84,357
D.T.P.	VALUE ENGINEERING COMPANY	N01CM67086	181,699
D.T.P.	WARF INSTITUTE, INC.	N01CM53770	38,061
	TOTAL		5,418,504

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY — FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II PROCUREMENT OF PRECLINICAL MATERIAL:		
D.T.P.	AGRICULTURE, DEPARTMENT OF	Y01CM40001	40,000
D.T.P.	ALDRICH CHEMICAL COMPANY, INC.	N01CM87202	138,343
O.D.	ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO60603	39,762
D.T.P.	ASH STEVENS, INC.	N01CM57012	184,399
D.T.P.	ASH STEVENS, INC.	N01CM87204	98,403
D.T.P.	ASSOCIATED BIOMEDIC SYSTEMS, INC.	N01CM02271	25,006
D.T.P.	BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223	5,499
D.T.P.	COLLABORATIVE RESEARCH, INC.	N01CM77071	9,427
D.T.P.	CORDOVA CHEMICAL COMPANY	N01CM87201	125,887
D.T.P.	HEM RESEARCH, INC.	N01CM67066	13,053
D.T.P.	ILLINOIS, UNIVERSITY OF	N01CM67090	18,098
D.T.P.	LITTON BIONETICS, INC.	N01CM12340	48,643
D.T.P.	LITTON BIONETICS, INC.	N01CM77134	363,571
D.T.P.	LITTON BIONETICS, INC.	N01CO75380	386,311
D.T.P.	MICROBIOLOGICAL ASSOCIATES	N01CM43793	127,500
D.T.P.	MIDWEST RESEARCH INSTITUTE	N01CM33722	10,540
D.T.P.	MONSANTO RESEARCH CORPORATION	N01CM67046	110,199
D.T.P.	PARKE, DAVIS AND COMPANY	N01CM87124	169,953
D.T.P.	PHARM-ECO	N01CM87200	129,112
D.T.P.	PURDUE UNIVERSITY	N01CM67091	18,866
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N01CM67089	29,102
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N01CM67121	128,000
D.T.P.	SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH	N01CM53820	18,092
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43762	[illegible],100
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM33723	16,174

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II PROCUREMENT OF PRECLINICAL MATERIAL:		
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM63776	128,000
D.T.P.	STARKS ASSOCIATES, INC.	N01CM53769	146,867
D.T.P.	STARKS ASSOCIATES, INC.	N01CM87203	217,624
D.T.P.	VETERANS ADMINISTRATION HOSPITAL	Y01CM60102	3,702
	TOTAL		2,753,233

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE III PHARMACOLOGY/TOXICOLOGY:		
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM53849	256,042
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM87163	660,912
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM43746	2,446,329
D.T.P.	BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223	16,498
CTEP	GEORGETOWN UNIVERSITY	N01CM67110	24,950
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM33708	157,908
A.P.	HAZLETON LABORATORIES, INC.	N01CM60125	32,400
D.T.P.	HEALTH RESEARCH, INC.	N01CM77118	49,200
A.P.	LABORATORY RESEARCH ENTERPRISES, INC.	N01CM60124	30,900
D.T.P.	MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM67064	75,580
A.P.	MARSHALL RESEARCH ANIMALS, INC.	N01CM60123	29,400
A.P.	MASON RESEARCH INSTITUTE	N01CM87164	96,581
D.T.P.	MEDICAL RESEARCH COUNCIL	N01CM87171	12,286
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM53828	5,226
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM87161	25,540
D.T.P.	OREGON STATE UNIVERSITY	N01CM23201	46,520
BCRC	PUBLIC HEALTH SERVICE	Y01CM40003	65,529
D.T.P.	SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM67065	95,621
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	46,000
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43780	209,592
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM57000	234,500
	TOTAL		4,617,514

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE III PROD. AND FORM. FOR CLINICAL TRIALS:		
D.T.P.	ALDRICH CHEMICAL COMPANY, INC.	N01CM87202	138,343
D.T.P.	ASH STEVENS, INC.	N01CM57012	61,466
D.T.P.	ASH STEVENS, INC.	N01CM87204	98,403
D.T.P.	BEN VENUE LABORATORIES, INC.	N01CM67109	1,034,955
D.T.P.	CORDOVA CHEMICAL COMPANY	N01CM87201	125,887
D.T.P.	FLOW LABORATORIES, INC.	N01CM67088	231,000
D.T.P.	IOWA, UNIVERSITY OF	N01CM43743	192,826
C.O.P.	LITTON BIONETICS, INC.	N01CM67067	6,550
D.T.P.	MICROBIOLOGICAL ASSOCIATES	N01CM43793	42,500
D.T.P.	MIDWEST RESEARCH INSTITUTE	N01CM33722	42,160
D.T.P.	MONSANTO RESEARCH CORPORATION	N01CM67046	991,795
D.T.P.	PARKE, DAVIS AND COMPANY	N01CM87124	169,953
D.T.P.	PHARM-ECO	N01CM87200	129,112
D.T.P.	PHILIPS ROXANE LABORATORIES, INC.	N01CM67053	107,675
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N01CM67121	32,000
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM33723	72,694
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM63776	32,000
D.T.P.	STARKS ASSOCIATES, INC.	N01CM53769	48,956
D.T.P.	STARKS ASSOCIATES, INC.	N01CM87203	217,624
	TOTAL		3,775,899

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	PRECLIN. BASIC RES. - BIOLOGICAL STUDIES:		
D.T.P.	AMERICAN TYPE CULTURE COLLECTION	N01CM87158	180,847
O.D.	ARTEK SYSTEMS CORPORATION	N01CM77128	10,500
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM53849	49,878
D.T.P.	ASSOCIATED BIOMEDIC SYSTEMS, INC.	N01CM02271	25,006
C.D.	ENERGY RES & DEV ADM	Y01CM40102	7,500
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM33708	118,431
C.O.P.	HAZLETON LABORATORIES, INC.	N01CM87156	183,490
D.T.P.	HEALTH RESEARCH, INC.	N01CM57039	104,515
D.T.P.	HEM RESEARCH, INC.	N01CM67066	13,053
O.D.	INSTITUT JULES BORDET	N01CM53840	16,380
D.T.P.	LITTON BIONETICS, INC.	N01CM12340	48,643
D.T.P.	LITTON BIONETICS, INC.	N01CM22062	107,005
C.O.P.	LITTON BIONETICS, INC.	N01CM67067	58,948
D.T.P.	LITTON BIONETICS, INC.	N01CM77134	363,571
C.O.P.	LITTON BIONETICS, INC.	N01CM87169	175,000
A.P.	MASON RESEARCH INSTITUTE	N01CM87164	18,815
D.T.P.	MIAMI, UNIVERSITY OF	N01CM57041	26,540
C.O.P.	MICROBIOLOGICAL ASSOCIATES	N01CM63810	50,000
BCRC	PUBLIC HEALTH SERVICE	Y01CM40003	102,974
D.T.P.	RES. FDN. OF STATE UNIVERSITY OF NEW YORK	N01CM53766	151,164
D.T.P.	SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH	N01CM53820	54,275
D.T.P.	SMALL BUSINESS ADMINISTRATION	N01CM43719	47,969
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	23,000
CTEP	WALTER REED ARMY MEDICAL CENTER	Y01CM80108	67,425

TOTAL	2,004,929

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY — FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	PRECLIN. BASIC RES. - TREATMENT STUDIES:		
O.D.	ARTEK SYSTEMS CORPORATION	N01CM77128	10,500
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM53765	337,500
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM57005	33,657
D.T.P.	CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM67081	90,783
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM57007	16,439
C.O.P.	HAZLETON LABORATORIES, INC.	N01CM87156	78,639
D.T.P.	HEALTH RESEARCH, INC.	N01CM77118	32,800
D.T.P.	IIT RESEARCH INSTITUTE	N01CM57004	22,923
C.O.P.	LITTON BIONETICS, INC.	N01CM87169	75,000
D.T.P.	MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM53826	96,809
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57003	67,802
C.O.P.	MASON RESEARCH INSTITUTE	N01CM67011	209,262
D.T.P.	MEDICAL RESEARCH COUNCIL	N01CM87171	8,191
C.D.	RADIOBIOLOGICAL INST., ORGAN. FOR HEALTH RES.	N01CM53763	73,151
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	874,000
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM57000	100,500
D.T.P.	VALUE ENGINEERING COMPANY	N01CM67086	72,680
D.T.P.	WARF INSTITUTE, INC.	N01CM53770	4,229
	TOTAL		2,204,865

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	PROGRAM MANAGEMENT:		
CTEP	AMERICAN COLLEGE OF RADIOLOGY	N01CM87175	148,547
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM33727	189,000
CTEP	BOWMAN GRAY SCHOOL OF MEDICINE OF WAKE FOREST	N01CM67054	4,188
CTEP	CLINICA NEUROCHIRURGICA DELL UNIVERSITA	N01CM67056	2,959
CTEP	CONTROL DATA CORPORATION	N01CM67107	5,000
CTEP	DUKE UNIVERSITY	N01CM67010	9,891
BCRC	HAZLETON LABORATORIES, INC.	N01CM67080	13,309
CTEP	INDIANA UNIVERSITY FOUNDATION	N01CM67057	5,259
CTEP	INFORMATION PLANNING ASSOC.	N01CM77104	150,000
O.D.	INSTITUT JULES BORDET	N01CM53840	40,950
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	9,800
CTEP	KENTUCKY, UNIVERSITY OF	N01CM67058	388
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	181,500
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57019	14,947
BCRC	MICROBIOLOGICAL ASSOCIATES	N01CM77131	5,999
CTEP	MONTEFIORE HOSPITAL	N01CM67061	10,350
A.P.	NATIONAL ACADEMY OF SCIENCES	N01CM53850	20,000
A.P.	NATIONAL ACADEMY OF SCIENCES	N01CM57013	25,000
CTEP	NEW YORK UNIVERSITY MEDICAL CENTER	N01CM67059	12,156
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM67060	4,849
BCRC	PUBLIC HEALTH SERVICE	Y01CM40003	18,723
BCRC	PUBLIC HEALTH SERVICE	Y01CM77132	17,500
O.D.	ROCHESTER, UNIVERSITY OF	N01CM77159	96,000
CTEP	SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	13,313
CTEP	TENNESSEE, UNIVERSITY OF	N01CM57021	10,797

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	PROGRAM MANAGEMENT:		
BCRC	VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE	N01CM33716	8,400
	TOTAL		1,018,825

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE I CLINICAL TRIALS:		
CTEP	CHILDREN'S HOSPITAL OF LOS ANGELES	N01CM43803	28,949
CTEP	GEORGETOWN UNIVERSITY	N01CM67110	37,426
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	7,350
CTEP	KANSAS, UNIVERSITY OF-MEDICAL CENTER	N01CM43802	27,826
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	363,000
CTEP	MAYO FOUNDATION	N01CM53838	39,657
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM43804	29,712
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM53837	71,998
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM53828	41,811
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM43801	43,750
	TOTAL		691,479

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE II CLINICAL TRIALS:		
CTEP	BOWMAN GRAY SCHOOL OF MEDICINE OF WAKE FOREST	N01CM67054	12,564
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM77151	16,348
CTEP	CLINICA NEUROCHIRURGICA DELL UNIVERSITA	N01CM67056	8,878
CTEP	DUKE UNIVERSITY	N01CM67010	29,674
CTEP	FRED HUTCHINSON CANCER RESEARCH CENTER	N01CM77148	31,222
CTEP	GEORGETOWN UNIVERSITY	N01CM67110	37,426
CTEP	HEALTH RESEARCH, INC.	N01CM43794	24,966
CTEP	HEALTH RESEARCH, INC.	N01CM53834	78,249
CTEP	INDIANA UNIVERSITY FOUNDATION	N01CM67057	15,776
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM33714	17,688
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM43726	41,965
C.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	7,350
CTEP	KENTUCKY, UNIVERSITY OF	N01CM67058	1,165
CTEP	LEEDS, UNIVERSITY OF	N01CM43727	16,450
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	1,234,200
CTEP	MAYO FOUNDATION	N01CM02066	22,969
CTEP	MAYO FOUNDATION	N01CM43796	54,300
CTEP	MAYO FOUNDATION	N01CM53772	189,446
CTEP	MAYO FOUNDATION	N01CM57044	306,116
CTEP	MAYO FOUNDATION	N01CM77150	9,279
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM53844	10,717
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57019	44,842
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57043	228,664
CTEP	MIAMI, UNIVERSITY OF	N01CM43800	64,309
CTEP	MONTEFIORE HOSPITAL	N01CM67061	31,051

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE II CLINICAL TRIALS:		
CTEP	NEW YORK UNIVERSITY MEDICAL CENTER	N01CM67059	36,468
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM53828	5,226
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM67060	14,546
CTEP	ONTARIO CANCER INSTITUTE	N01CM77152	37,751
CTEP	SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	39,939
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM67037	190,160
CTEP	TENNESSEE, UNIVERSITY OF	N01CM57021	32,390
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM57042	362,027
CTEP	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM77149	28,983
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM77153	26,166
CTEP	VANDERBILT UNIVERSITY MEDICAL CENTER	N01CM77122	22,814
CTEP	WAYNE STATE UNIVERSITY	N01CM67105	191,651
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM43797	43,470
	TOTAL		3,567,205

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1978
AS OF 05/31/78

PHASE III CLINICAL TRIALS:

AREA	CONTRACT	#	DOLLAR LEVEL
CTEP	BOWMAN GRAY SCHOOL OF MEDICINE OF WAKE FOREST	N01CM67054	25,127
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM77151	49,043
CTEP	CLINICA NEUROCHIRURGICA DELL UNIVERSITA	N01CM67056	17,756
CTEP	DUKE UNIVERSITY	N01CM67010	59,348
CTEP	FRED HUTCHINSON CANCER RESEARCH CENTER	N01CM77148	93,667
CTEP	GEORGETOWN UNIVERSITY	N01CM67110	74,851
CTEP	HEALTH RESEARCH, INC.	N01CM67117	65,508
CTEP	INDIANA UNIVERSITY FOUNDATION	N01CM67057	31,553
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM33714	41,272
CTEP	KENTUCKY, UNIVERSITY OF	N01CM67058	2,330
C.O.P.	MAKERERE UNIVERSITY COLLEGE COUNCIL	N01CM71343	25,000
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	943,800
CTEP	MAYO FOUNDATION	N01CM02066	7,656
CTEP	MAYO FOUNDATION	N01CM43783	49,600
CTEP	MAYO FOUNDATION	N01CM57044	250,459
CTEP	MAYO FOUNDATION	N01CM67123	112,488
CTEP	MAYO FOUNDATION	N01CM77150	27,836
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM53844	56,263
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57019	89,683
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57043	152,442
CTEP	MIAMI, UNIVERSITY OF	N01CM43800	101,058
CTEP	MONTEFIORE HOSPITAL	N01CM67061	62,102
CTEP	NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	38,100
CTEP	NEW YORK UNIVERSITY MEDICAL CENTER	N01CM67059	72,937
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM67060	29,093

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	#	DOLLAR LEVEL
	FOR FISCAL YEAR 1978 AS OF 05/31/78		
	PHASE III CLINICAL TRIALS:		
CTEP	ONTARIO CANCER INSTITUTE	N01CM77152	113,254
CTEP	PITTSBURGH, UNIVERSITY OF	N01CB23876	830,725
CTEP	SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	79,878
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM67037	155,585
CTEP	TENNESSEE, UNIVERSITY OF	N01CM57021	64,781
CTEP	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM33710	192,831
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM57042	296,204
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM67026	27,617
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM67116	46,369
CTEP	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM77149	86,950
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM77153	78,499
CTEP	VANDERBILT UNIVERSITY MEDICAL CENTER	N01CM77122	68,443
BCRC	VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE	N01CM33716	33,600
C.O.P.	VETERANS ADMINISTRATION	Y01CM70106	939,927
CTEP	VETERANS ADMINISTRATION	Y01CM70107	512,819
CTEP	WAYNE STATE UNIVERSITY	N01CM67105	156,805
	TOTAL		6,163,259

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE IV CLINICAL TRIALS (COMBINED MODALITY):		
CTEP	ALBANY MEDICAL COLLEGE OF UNION UNIVERSITY	NO1CM57032	112,522
CTEP	AMERICAN COLLEGE OF RADIOLOGY	NO1CM87219	210,070
CTEP	CALIFORNIA, UNIVERSITY OF	NO1CM67097	118,000
CTEP	CALIFORNIA, UNIVERSITY OF	NO1CM77151	16,348
CTEP	CHICAGO, UNIVERSITY OF	NO1CM67095	134,480
CTEP	CINCINNATI, UNIVERSITY OF	NO1CM87222	44,319
CTEP	FRED HUTCHINSON CANCER RESEARCH CENTER	NO1CM77148	31,222
CTEP	GEORGETOWN UNIVERSITY	NO1CM67094	108,609
CTEP	HEALTH RESEARCH, INC.	NO1CM43782	17,240
CTEP	HEALTH RESEARCH, INC.	NO1CM43794	41,611
CTEP	HEALTH RESEARCH, INC.	NO1CM57034	84,807
CTEP	HEALTH RESEARCH, INC.	NO1CM67117	65,508
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	NO1CM33714	58,961
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	NO1CM43726	62,947
BCRC	MARYLAND, UNIVERSITY OF	NO1CM43748	907,500
CTEP	MARYLAND, UNIVERSITY OF	NO1CM87223	83,740
CTEP	MAYO FOUNDATION	NO1CM43783	55,800
CTEP	MAYO FOUNDATION	NO1CM43796	90,500
CTEP	MAYO FOUNDATION	NO1CM57033	121,780
CTEP	MAYO FOUNDATION	NO1CM67123	112,488
CTEP	MAYO FOUNDATION	NO1CM77150	9,279
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	NO1CM87224	81,662
CTEP	MIAMI, UNIVERSITY OF	NO1CM43800	18,374
CTEP	MIAMI, UNIVERSITY OF	NO1CM67093	106,843
CTEP	MICHIGAN, UNIVERSITY OF	NO1CM87225	133,680

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE IV CLINICAL TRIALS (COMBINED MODALITY):		
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM53841	166,429
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM67096	121,721
CTEP	NORTHERN CALIFORNIA CANCER PROGRAM	N01CM87154	177,314
CTEP	ONTARIO CANCER INSTITUTE	N01CM77152	37,751
CTEP	PITTSBURGH, UNIVERSITY OF	N01CM77177	716,506
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM43781	55,846
CTEP	SOUTH FLORIDA, UNIVERSITY OF	N01CM87220	70,002
CTEP	SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM53842	33,300
[illegible]	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM67026	27,617
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM67116	46,369
CTEP	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM77149	28,983
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM77153	26,166
CTEP	TEXAS, UNIVERSITY OF, MEDICAL BRANCH	N01CM87221	95,072
CTEP	VANDERBILT UNIVERSITY MEDICAL CENTER	N01CM77122	22,814
C.O.P.	VETERANS ADMINISTRATION	Y01CM70106	939,927
CTEP	VETERANS ADMINISTRATION	Y01CM70107	512,819
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM43797	72,450
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM53843	94,500
	TOTAL		6,074,376

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS - OTHER RESEARCH:		
TEP	CONTROL DATA CORPORATION	N01CM67107	45,000
CTEP	HEALTH RESEARCH, INC.	N01CM43794	16,644
CTEP	MAYO FOUNDATION	N01CM43783	18,600
CTEP	MAYO FOUNDATION	N01CM43796	36,200
CTEP	MAYO FOUNDATION	N01CM53838	9,914
CTEP	MAYO FOUNDATION	N01CM57033	30,445
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM53837	17,999
C.O.P.	VETERANS ADMINISTRATION	Y01CM70106	469,963
CTEP	VETERANS ADMINISTRATION	Y01CM70107	256,409
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM43797	28,980
	TOTAL		930,154

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS SUPPORTIVE RESEARCH:		
C.O.P.	ABBOTT LABORATORIES	NO1CM43704	28,064
C.O.P.	CALIFORNIA, UNIVERSITY OF	NO1CM02092	392,000
C.D.	CALIFORNIA, UNIVERSITY OF	NO1HB62971	66,578
C.O.P.	COMMUNITY BLOOD & PLASMA SERVICE	NO1CL42150	602,405
O.D.	ENERGY RES & DEV ADM	YO1CM40102	67,500
CTEP	FRONTIER SCIENCE & TECHNOLOGY RES. FDN., INC.	NO1CM77136	199,952
CTEP	GEORGETOWN UNIVERSITY	NO1CM67110	74,851
CTEP	GEORGETOWN UNIVERSITY	NO1CM87194	174,985
BCRC	HAZLETON LABORATORIES, INC.	NO1CM67080	430,327
CTEP	HEALTH RESEARCH, INC.	NO1CM43782	1,916
CTEP	INFORMATION MANAGEMENT SERVICES, INC.	NO1CP71010	30,988
C.D.	IOWA, UNIVERSTIY OF	NO1HB62973	59,824
D.T.P.	KANSAS, UNIVERSITY OF-MEDICAL CENTER	NO1CM12184	50,000
D.T.P.	KENTUCKY, UNIVERSITY OF	NO1CM67038	159,075
CTEP	LEEDS, UNIVERSITY OF	NO1CM43727	16,450
BCRC	MICROBIOLOGICAL ASSOCIATES	NO1CM77131	79,701
C.D.	MINNESOTA, UNIVERSITY OF	NO1HB62972	59,032
D.T.P.	MISSOURI, UNIVERSITY OF	NO1CM12323	55,000
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	NO1CM53841	18,492
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	NO1CM67096	13,525
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	NO1CM87161	76,619
BCRC	PUBLIC HEALTH SERVICE	YO1CM77132	332,500
CTEP	SOUTHERN CALIFORNIA, UNIVERSITY OF	NO1CM53842	3,700
D.T.P.	SOUTHERN CALIFORNIA, UNIVERSITY OF	NO1CM67065	31,874
C.O.P.	TEXAS, UNIVERSITY OF	NO1CM53832	650,000

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1978
AS OF 05/31/78

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS SUPPORTIVE RESEARCH:		
.D.	WASHINGTON UNIVERSITY	N01HB62970	53,465
IEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM53843	10,500
	TOTAL		3,739,323

TABLE III

ANALYSIS OF DIVISION OF CANCER TREATMENT

ACTIVITIES BY
ACTIVE CONTRACTS

FOR FISCAL YEAR 1978
AS OF 05/31/78

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ABBOTT LABORATORIES	N01CM43704 C.O.P.			28,064
CLINICAL TRIALS SUPPORTIVE RESEARCH LEUK.-ACUTE GRAN. BLOOD PRODUCTS		*100.00*	28,064	
AGRICULTURE, DEPARTMENT OF	Y01CM40001 D.T.P.			400,000
ACQUISITION OF MATERIALS		* 90.00*	360,000	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		85.00	340,000	
DATA PROCESSING AND SUPPORT		5.00	20,000	
PROCUREMENT OF PRECLINICAL MATERIAL PLANT PRODUCTS		* 10.00*	40,000	
ALABAMA, UNIVERSITY OF	N01CM67083 A.P.			10,048
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	2,010	
BASIC SCREEN ANIMALS		* 55.00*	5,526	
VERIFICATION SCREEN ANIMALS		* 25.00*	2,512	
ALBANY MEDICAL COLLEGE OF UNION	N01CM57032 CTEP			112,522
PHASE IV CLINICAL TRIALS		*100.00*	112,522	
COLORECTAL SURG.		30.00	33,757	
COLORECTAL RAD.		10.00	11,252	
COLORECTAL CHEM.		25.00	28,131	
COLORECTAL IMM.		25.00	28,131	
COLORECTAL SUPPORTIVE CARE		10.00	11,252	
ALDRICH CHEMICAL COMPANY, INC.	N01CM87202 D.T.P.			276,685
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 50.00*	138,343	
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	138,343	
AMERICAN COLLEGE OF RADIOLOGY	N01CM87175 CTEP			148,547
PROGRAM MANAGEMENT		*100.00*	148,547	
COMMUNICATION AND EDUCATION		40.00	59,419	
RESOURCE DEVELOPMENT		20.00	29,709	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
DATA PROCESSING AND SUPPORT		40.00	59,419	
AMERICAN COLLEGE OF RADIOLOGY	N01CM87219 CTEP			210,070
PHASE IV CLINICAL TRIALS		*100.00*	210,070	
HEAD AND NECK SURG.		30.00	63,021	
HEAD AND NECK RAD.		30.00	63,021	
HEAD AND NECK CHEM.		20.00	42,014	
HEAD AND NECK SUPPORTIVE CARE		20.00	42,014	
AMERICAN TYPE CULTURE COLLECTION	N01CM87158 D.T.P.			180,847
BIOLOGICAL STUDIES		*100.00*	180,847	
BLOOD PRODUCTS		40.00	72,339	
TISSUE CULTURE CELLS		60.00	108,508	
ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO60603 O.D.			397,621
ACQUISITION OF MATERIALS		* 48.00*	190,858	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT				
BASIC SCREEN		* 12.00*	47,715	
PRIMARY SCREENING, IN VITRO		3.00	11,929	
ANIMALS		9.00	35,786	
VERIFICATION SCREEN		* 30.00*	119,286	
ANIMALS				
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	39,762	
FERMENTATION/ANTIBIOTICS				
ARS/SPRAGUE-DAWLEY	N01CM53813 A.P.			1,070,000
ACQUISITION OF MATERIALS		* 20.00*	214,000	
ANIMALS				
BASIC SCREEN		* 55.00*	588,500	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	267,500	
ANIMALS				
ARS/SPRAGUE-DAWLEY	N01CM53846 A.P.			251,836
ACQUISITION OF MATERIALS		* 20.00*	50,367	
ANIMALS				
BASIC SCREEN		* 55.00*	138,510	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	62,959	
ANIMALS				
[illegible]TEK SYSTEMS CORPORATION	N01CM77128 O.D.			21,000
BIOLOGICAL STUDIES		* 50.00*	10,500	
CELL BIOLOGY		15.00	3,150	
CELL KINETICS		35.00	7,350	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
TREATMENT STUDIES		* 50.00*	10,500	
CHEMOTHERAPY				
ARTHUR D. LITTLE, INC.	N01CM67047 CTEP			129,771
VERIFICATION SCREEN		*100.00*	129,771	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL				
ARTHUR D. LITTLE, INC.	N01CM53849 D.T.P.			332,522
BASIC SCREEN		* 8.00*	26,602	
TUMOR BANK				
PHARMACOLOGY/TOXICOLOGY		* 77.00*	256,042	
PHARMACOLOGY				
BIOLOGICAL STUDIES		* 15.00*	49,878	
BIOCHEMISTRY				
ARTHUR D. LITTLE, INC.	N01CM57006 D.T.P.			616,882
ACQUISITION OF MATERIALS		* 3.00*	18,506	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 72.00*	444,155	
PRIMARY SCREENING, IN VIVO		58.00	357,792	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		14.00	86,363	
VERIFICATION SCREEN		* 25.00*	154,221	
DETAILED DRUG EVALUATION, IN VIVO				
ARTHUR D. LITTLE, INC.	N01CM33727 D.T.P.			189,000
PROGRAM MANAGEMENT		*100.00*	189,000	
APP. MATH/DATA ANAL.				
ARTHUR D. LITTLE, INC.	N01CM53765 D.T.P.			750,000
BASIC SCREEN		* 55.00*	412,500	
IN VIVO ANALOG SCR.				
TREATMENT STUDIES		* 45.00*	337,500	
COMB. MODAL. THERAPY		45.00	337,500	
COMB. MODAL. THERAPY - RAD.		20.00	150,000	
COMB. MODAL. THERAPY - CHEM.		25.00	187,500	
ARTHUR D. LITTLE, INC.	N01CM87163 D.T.P.			660,912
PHARMACOLOGY/TOXICOLOGY		*100.00*	660,912	
PHARMACOLOGY		80.00	528,730	
RADIOAUTOG/ANAL		20.00	132,182	
ASH STEVENS, INC.	N01CM87204 D.T.P.			196,805
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	98,403	
SYNTHETICS				

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	98,403	
ASH STEVENS, INC.	N01CM57012 D.T.P.			245,865
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 75.00*	184,399	
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 25.00*	61,466	
ASSOCIATED BIOMEDIC SYSTEMS, INC.	N01CM02271 D.T.P.			250,055
ACQUISITION OF MATERIALS HUMAN BLOOD		* 80.00*	200,044	
PROCUREMENT OF PRECLINICAL MATERIAL ANALYTICAL AND QUALITY CONTROL		* 10.00*	25,006	
BIOLOGICAL STUDIES MOLECULAR BIOLOGY		* 10.00*	25,006	
BATTELLE MEMORIAL INSTITUTE	N01CM43746 D.T.P.			2,446,329
PHARMACOLOGY/TOXICOLOGY		*100.00*	2,446,329	
PROTOCOL TOXICITY STUDIES		50.00	1,223,165	
SPECIAL TOXICITY STUDIES		34.00	831,752	
ANIMALS		1.00	24,463	
DATA PROCESSING AND SUPPORT		15.00	366,949	
BATTELLE MEMORIAL INSTITUTE	N01CM67099 D.T.P.			472,500
BASIC SCREEN		*100.00*	472,500	
PRIMARY SCREENING, IN VIVO		75.00	354,375	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		25.00	118,125	
BATTELLE MEMORIAL INSTITUTE	N01CM57005 D.T.P.			673,142
ACQUISITION OF MATERIALS BIOASSAY OF NATURAL PRODUCTS		* 15.00*	100,971	
BASIC SCREEN		* 55.00*	370,228	
PRIMARY SCREENING, IN VIVO		50.00	336,571	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	33,657	
VERIFICATION SCREEN DETAILED DRUG EVALUATION, IN VIVO		* 25.00*	168,286	
TREATMENT STUDIES CHEMOTHERAPY		* 5.00*	33,657	
BELLAIRE ACRES	N01CM50583 A.P.			229,320
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	45,864	
BASIC SCREEN ANIMALS		* 55.00*	126,126	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
VERIFICATION SCREEN		* 25.00*	57,330	
ANIMALS				
BEN VENUE LABORATORIES, INC.	N01CM67109 D.T.P.			1,149,950
FORMULATION		* 10.00*	114,995	
DEVEL. OF EXP. FORMULATIONS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 90.00*	1,034,955	
FORMULATION		75.00	862,463	
ANALYTICAL AND QUALITY CONTROL		15.00	172,493	
BOWMAN GRAY SCHOOL OF MEDICINE OF WAKE	N01CM67054 CTEP			41,879
PROGRAM MANAGEMENT		* 10.00*	4,188	
ADMINISTRATION				
PHASE II CLINICAL TRIALS		* 30.00*	12,564	
CNS TUMORS CHEM.				
PHASE III CLINICAL TRIALS		* 60.00*	25,127	
CNS TUMORS RAD.		30.00	12,564	
CNS TUMORS CHEM.		30.00	12,564	
BRISTOL-MYERS COMPANY	N01CM77138 D.T.P.			260,000
ACQUISITION OF MATERIALS		*100.00*	260,000	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT				
BRISTOL-MYERS COMPANY	N01CM77147 D.T.P.			333,000
ACQUISITION OF MATERIALS		* 85.00*	283,050	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	276,390	
DATA PROCESSING AND SUPPORT		2.00	6,660	
BASIC SCREEN		* 15.00*	49,950	
PRIMARY SCREENING, IN VITRO		10.00	33,300	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	16,650	
BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223 D.T.P.			109,985
ACQUISITION OF MATERIALS		* 80.00*	87,988	
NEW AGENT PROCUREMENT, SYNTHETICS				
PROCUREMENT OF PRECLINICAL MATERIAL		* 5.00*	5,499	
RADIOLABELED MATERIALS				
PHARMACOLOGY/TOXICOLOGY		* 15.00*	16,498	
METABOLITE ISOLATION				
CALIFORNIA, UNIVERSITY OF	N01CM02092 C.O.P.			392,000
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	392,000	
NON-SPECIFIC TRANSFUSION RES.				

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
CALIFORNIA, UNIVERSITY OF	N01CM67097 CTEP			118,000
PHASE IV CLINICAL TRIALS		*100.00*	118,000	
COLORECTAL SURG.		20.00	23,600	
COLORECTAL RAD.		15.00	17,700	
COLORECTAL CHEM.		45.00	53,100	
COLORECTAL IMM.		20.00	23,600	
CALIFORNIA, UNIVERSITY OF	N01CM77151 CTEP			81,739
PHASE II CLINICAL TRIALS		* 20.00*	16,348	
LUNG RAD.		10.00	8,174	
LUNG CHEM.		10.00	8,174	
PHASE III CLINICAL TRIALS		* 60.00*	49,043	
LUNG RAD.		30.00	24,522	
LUNG CHEM.		30.00	24,522	
PHASE IV CLINICAL TRIALS		* 20.00*	16,348	
LUNG RAD.		10.00	8,174	
LUNG CHEM.		10.00	8,174	
CALIFORNIA, UNIVERSITY OF	N01HB62971 O.D.			66,578
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	66,578	
OTHER TRANSFUSION RES.				
CATHOLIC MEDICAL CTR OF BROOKLYN AND	N01CM67081 D.T.P.			453,914
BASIC SCREEN		* 40.00*	181,566	
PRIMARY SCREENING, IN VIVO		18.00	81,705	
ANIMALS		22.00	99,861	
VERIFICATION SCREEN		* 40.00*	181,566	
DETAILED DRUG EVALUATION, IN VIVO		18.00	81,705	
ANIMALS		22.00	99,861	
TREATMENT STUDIES		* 20.00*	90,783	
COMB. MODAL. THERAPY		20.00	90,783	
COMB. MODAL. THERAPY - SURG.		9.00	40,852	
COMB. MODAL. THERAPY - CHEM.		9.00	40,852	
COMB. MODAL. THERAPY - IMM.		2.00	9,078	
CHARLES RIVER BREEDING LABS.	N01CM53848 A.P.			230,050
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	46,010	
BASIC SCREEN ANIMALS		* 55.00*	126,528	
VERIFICATION SCREEN ANIMALS		* 25.00*	57,513	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
CHARLES RIVER BREEDING LABS.	N01CM50598 A.P.			260,520
ACQUISITION OF MATERIALS		* 20.00*	52,104	
ANIMALS				
BASIC SCREEN		* 55.00*	143,286	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	65,130	
ANIMALS				
CHARLES RIVER BREEDING LABS.	N01CM77141 A.P.			1,560,382
ACQUISITION OF MATERIALS		* 20.00*	312,076	
ANIMALS				
BASIC SCREEN		* 55.00*	858,210	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	390,096	
ANIMALS				
CHEMICAL ABSTRACTS SERVICE	N01CM43722 D.T.P.			449,600
ACQUISITION OF MATERIALS		*100.00*	449,600	
DATA PROCESSING AND SUPPORT				
CHESTER BEATTY RESEARCH INSTITUTE	N01CM43736 D.T.P.			104,000
ACQUISITION OF MATERIALS		* 50.00*	52,000	
NEW AGENT PROCUREMENT, SYNTHETICS		40.00	41,600	
DATA PROCESSING AND SUPPORT		10.00	10,400	
VERIFICATION SCREEN		* 50.00*	52,000	
DETAILED DRUG EVALUATION, IN VITRO		5.00	5,200	
DETAILED DRUG EVALUATION, IN VIVO		20.00	20,800	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		25.00	26,000	
CHICAGO, UNIVERSITY OF	N01CM67095 CTEP			134,480
PHASE IV CLINICAL TRIALS		*100.00*	134,480	
COLORECTAL SURG.		20.00	26,896	
COLORECTAL RAD.		15.00	20,172	
COLORECTAL CHEM.		45.00	60,516	
COLORECTAL IMM.		20.00	26,896	
CHILDREN'S HOSPITAL OF LOS ANGELES	N01CM43803 CTEP			28,949
PHASE I CLINICAL TRIALS		*100.00*	28,949	
LEUK.-ACUTE GRAN. CHEM.		30.00	8,685	
LEUK.-ACUTE LYMPH. CHEM.		30.00	8,685	
NEUROBLASTOMA CHEM.		10.00	2,895	
RHABDOMYOSARCOMA CHEM.		20.00	5,790	
WILMS' TUMOR CHEM.		10.00	2,895	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
CINCINNATI, UNIVERSITY OF		N01CM87222 CTEP			44,319
PHASE IV CLINICAL TRIALS			*100.00*	44,319	
HEAD AND NECK	SURG.		30.00	13,296	
HEAD AND NECK	RAD.		30.00	13,296	
HEAD AND NECK	CHEM.		20.00	8,864	
HEAD AND NECK	SUPPORTIVE CARE		20.00	8,864	
CLINICA NEUROCHIRURGICA DELL		N01CM67056 CTEP			29,594
PROGRAM MANAGEMENT ADMINISTRATION			* 10.00*	2,959	
PHASE II CLINICAL TRIALS			* 30.00*	8,878	
CNS TUMORS	RAD.				
PHASE III CLINICAL TRIALS			* 60.00*	17,756	
CNS TUMORS	RAD.		30.00	8,878	
CNS TUMORS	CHEM.		30.00	8,878	
COLLABORATIVE RESEARCH, INC.		N01CM77071 D.T.P.			94,271
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS			* 90.00*	84,844	
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS			* 10.00*	9,427	
COMMUNITY BLOOD & PLASMA SERVICE		N01CL42150 C.O.P.			602,405
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	602,405	
NON-SPECIFIC	BLOOD PRODUCTS				
CONTROL DATA CORPORATION		N01CM67107 CTEP			50,000
PROGRAM MANAGEMENT ADMINISTRATION			* 10.00*	5,000	
OTHER CLINICAL TRIALS RESEARCH			* 90.00*	45,000	
CNS TUMORS	DATA PROC. & SUPP.				
CORDOVA CHEMICAL COMPANY		N01CM87201 D.T.P.			251,774
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS			* 50.00*	125,887	
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS			* 50.00*	125,887	
DUKE UNIVERSITY		N01CM67010 CTEP			98,913
PROGRAM MANAGEMENT ADMINISTRATION			* 10.00*	9,891	
PHASE II CLINICAL TRIALS			* 30.00*	29,674	
CNS TUMORS	RAD.				

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PHASE III CLINICAL TRIALS			* 60.00*	59,348	
CNS TUMORS	RAD.		30.00	29,674	
CNS TUMORS	CHEM.		30.00	29,674	
NERGY RES & DEV ADM		Y01CM10056 D.T.P.			30,000
VERIFICATION SCREEN			*100.00*	30,000	
DETAILED DRUG EVALUATION, IN VITRO			70.00	21,000	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL			30.00	9,000	
ENERGY RES & DEV ADM		Y01CM40102 O.D.			75,000
BIOLOGICAL STUDIES			* 10.00*	7,500	
CELL KINETICS					
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 90.00*	67,500	
NON-SPECIFIC	CELL KINETICS				
FLOW LABORATORIES, INC.		N01CM50571 A.P.			229,320
ACQUISITION OF MATERIALS			* 20.00*	45,864	
ANIMALS					
BASIC SCREEN			* 55.00*	126,126	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	57,330	
ANIMALS					
FLOW LABORATORIES, INC.		N01CM67088 D.T.P.			231,000
PROD. AND FORM. FOR CLINICAL TRIALS			*100.00*	231,000	
STOR.&DIST. OF DRUGS					
FRED HUTCHINSON CANCER RESEARCH		N01CM77148 CTEP			156,111
PHASE II CLINICAL TRIALS			* 20.00*	31,222	
LUNG	RAD.		10.00	15,611	
LUNG	CHEM.		10.00	15,611	
PHASE III CLINICAL TRIALS			* 60.00*	93,667	
LUNG	RAD.		30.00	46,833	
LUNG	CHEM.		30.00	46,833	
PHASE IV CLINICAL TRIALS			* 20.00*	31,222	
LUNG	RAD.		10.00	15,611	
LUNG	CHEM.		10.00	15,611	
FRONTIER SCIENCE & TECHNOLOGY RES.		N01CM77136 CTEP			199,952
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	199,952	
LUNG	DATA PROC. & SUPP.				
GEORGETOWN UNIVERSITY		N01CM67094 CTEP			108,609
PHASE IV CLINICAL TRIALS			*100.00*	108,609	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
COLORECTAL	SURG.		20.00	21,722	
COLORECTAL	RAD.		15.00	16,291	
COLORECTAL	CHEM.		45.00	48,874	
COLORECTAL	IMM.		20.00	21,722	
GEORGETOWN UNIVERSITY		N01CM67110 CTEP			249,504
PHARMACOLOGY/TOXICOLOGY PHARMACOLOGY			* 10.00*	24,950	
PHASE I CLINICAL TRIALS			* 15.00*	37,426	
COLORECTAL	CHEM.		5.00	12,475	
PANCREATIC	CHEM.		5.00	12,475	
STOMACH	CHEM.		5.00	12,475	
PHASE II CLINICAL TRIALS			* 15.00*	37,426	
COLORECTAL	CHEM.		5.00	12,475	
PANCREATIC	CHEM.		5.00	12,475	
STOMACH	CHEM.		5.00	12,475	
PHASE III CLINICAL TRIALS			* 30.00*	74,851	
COLORECTAL	SURG.		2.00	4,990	
COLORECTAL	RAD.		2.00	4,990	
COLORECTAL	CHEM.		2.00	4,990	
COLORECTAL	IMM.		2.00	4,990	
COLORECTAL	SUPPORTIVE CARE		2.00	4,990	
PANCREATIC	SURG.		2.00	4,990	
PANCREATIC	RAD.		2.00	4,990	
PANCREATIC	CHEM.		2.00	4,990	
PANCREATIC	IMM.		2.00	4,990	
PANCREATIC	SUPPORTIVE CARE		2.00	4,990	
STOMACH	SURG.		2.00	4,990	
STOMACH	RAD.		2.00	4,990	
STOMACH	CHEM.		2.00	4,990	
STOMACH	IMM.		2.00	4,990	
STOMACH	SUPPORTIVE CARE		2.00	4,990	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 30.00*	74,851	
COLORECTAL	NUTRITION		10.00	24,950	
PANCREATIC	NUTRITION		10.00	24,950	
STOMACH	NUTRITION		10.00	24,950	
GEORGETOWN UNIVERSITY		N01CM87194 CTEP			174,985
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	174,985	
NON-SPECIFIC	OTHER				
GRACE, W.R.		N01CM67074 D.T.P.			200,000
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, MARINE/ARTHROPODS			*100.00*	200,000	
HARLAN INDUSTRIES		N01CM67079 A.P.			172,420
BASIC SCREEN			*100.00*	172,420	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ANIMALS					
HARLAN INDUSTRIES		N01CM50591 A.P.			237,120
ACQUISITION OF MATERIALS ANIMALS			* 20.00*	47,424	
BASIC SCREEN ANIMALS			* 55.00*	130,416	
VERIFICATION SCREEN ANIMALS			* 25.00*	59,280	
HARLAN INDUSTRIES		N01CM77168 A.P.			309,190
ACQUISITION OF MATERIALS ANIMALS			* 20.00*	61,838	
BASIC SCREEN ANIMALS			* 55.00*	170,055	
VERIFICATION SCREEN ANIMALS			* 25.00*	77,298	
HAWAII, UNIVERSITY OF		N01CM33747 D.T.P.			86,360
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, PLANT PRODUCTS			*100.00*	86,360	
HAZLETON LABORATORIES, INC.		N01CM60125 A.P.			32,400
PHARMACOLOGY/TOXICOLOGY ANIMALS			*100.00*	32,400	
HAZLETON LABORATORIES, INC.		N01CM67080 BCRC			443,636
PROGRAM MANAGEMENT			* 3.00*	13,309	
ADMINISTRATION			1.00	4,436	
DATA PROCESSING AND SUPPORT			2.00	8,873	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 97.00*	430,327	
LEUK.-ACUTE GRAN.	MICROBIOLOGY		33.00	146,400	
LEUK.-ACUTE LYMPH.	MICROBIOLOGY		5.00	22,182	
LEUK.-CHRON. GRAN.	MICROBIOLOGY		10.00	44,364	
LEUK.-CHRON LYMPH.	MICROBIOLOGY		5.00	22,182	
LYMPHOMA-HISTIOCYT	MICROBIOLOGY		2.00	8,873	
LYMPHOMA-HODGKIN'S	MICROBIOLOGY		10.00	44,364	
LYMPHOMA-LYMPHOCYT	MICROBIOLOGY		3.00	13,309	
LYMPHOMA-MYCO.FUNG	MICROBIOLOGY		4.00	17,745	
MYELOMA	MICROBIOLOGY		4.00	17,745	
CNS TUMORS	MICROBIOLOGY		5.00	22,182	
COLORECTAL	MICROBIOLOGY		6.00	26,618	
KIDNEY	MICROBIOLOGY		5.00	22,182	
LUNG	MICROBIOLOGY		3.00	13,309	
MELANOMA	MICROBIOLOGY		2.00	8,873	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
HAZLETON LABORATORIES, INC.	N01CM87156 C.O.P.			262,129
BIOLOGICAL STUDIES		* 70.00*	183,490	
BIOCHEMISTRY		20.00	52,426	
CELL KINETICS		20.00	52,426	
MARKERS		10.00	26,213	
NUTRITION		20.00	52,426	
TREATMENT STUDIES		* 30.00*	78,639	
SURGERY		10.00	26,213	
RADIOTHERAPY		10.00	26,213	
CHEMOTHERAPY		10.00	26,213	
HAZLETON LABORATORIES, INC.	N01CM33708 D.T.P.			394,770
ACQUISITION OF MATERIALS		* 20.00*	78,954	
ANIMALS				
VERIFICATION SCREEN		* 10.00*	39,477	
ANIMALS				
PHARMACOLOGY/TOXICOLOGY		* 40.00*	157,908	
PHARMACOLOGY		10.00	39,477	
SPECIAL TOXICITY STUDIES		30.00	118,431	
BIOLOGICAL STUDIES		* 30.00*	118,431	
MARKERS				
HAZLETON LABORATORIES, INC.	N01CM57007 D.T.P.			547,966
ACQUISITION OF MATERIALS		* 15.00*	82,195	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 69.00*	378,097	
PRIMARY SCREENING, IN VIVO		65.00	356,178	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		4.00	21,919	
VERIFICATION SCREEN		* 13.00*	71,236	
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES		* 3.00*	16,439	
CHEMOTHERAPY				
HEALTH RESEARCH, INC.	N01CM43794 CTEP			83,221
PHASE II CLINICAL TRIALS		* 30.00*	24,966	
PANCREATIC CHEM.				
PHASE IV CLINICAL TRIALS		* 50.00*	41,611	
PANCREATIC SURG.		5.00	4,161	
PANCREATIC RAD.		20.00	16,644	
PANCREATIC CHEM.		25.00	20,805	
OTHER CLINICAL TRIALS RESEARCH		* 20.00*	16,644	
PANCREATIC DATA PROC. & SUPP.				
HEALTH RESEARCH, INC.	N01CM53834 CTEP			78,249
PHASE II CLINICAL TRIALS		*100.00*	78,249	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
BREAST	CHEM.				
HEALTH RESEARCH, INC.		N01CM77101 A.P.			90,267
ACQUISITION OF MATERIALS			* 20.00*	18,053	
ANIMALS					
BASIC SCREEN			* 55.00*	49,647	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	22,567	
ANIMALS					
HEALTH RESEARCH, INC.		N01CM67117 CTEP			131,016
PHASE III CLINICAL TRIALS			* 50.00*	65,508	
OVARIAN	SURG.		15.00	19,652	
OVARIAN	RAD.		15.00	19,652	
OVARIAN	CHEM.		20.00	26,203	
PHASE IV CLINICAL TRIALS			* 50.00*	65,508	
OVARIAN	SURG.		15.00	19,652	
OVARIAN	RAD.		15.00	19,652	
OVARIAN	CHEM.		20.00	26,203	
HEALTH RESEARCH, INC.		N01CM43782 CTEP			19,156
PHASE IV CLINICAL TRIALS			* 90.00*	17,240	
STOMACH	SURG.		25.00	4,789	
STOMACH	RAD.		25.00	4,789	
STOMACH	CHEM.		40.00	7,662	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	1,916	
STOMACH	MARKERS				
HEALTH RESEARCH, INC.		N01CM57034 CTEP			84,807
PHASE IV CLINICAL TRIALS			*100.00*	84,807	
COLORECTAL	SURG.		20.00	16,961	
COLORECTAL	RAD.		15.00	12,721	
COLORECTAL	CHEM.		45.00	38,163	
COLORECTAL	IMM.		20.00	16,961	
HEALTH RESEARCH, INC.		N01CM77118 D.T.P.			164,000
FORMULATION			* 20.00*	32,800	
LIPOSOME ENCAPS.					
VERIFICATION SCREEN			* 30.00*	49,200	
DETAILED DRUG EVALUATION, IN VITRO			10.00	16,400	
DETAILED DRUG EVALUATION, IN VIVO			15.00	24,600	
DATA PROCESSING AND SUPPORT			5.00	8,200	
PHARMACOLOGY/TOXICOLOGY			* 30.00*	49,200	
PHARMACOLOGY			15.00	24,600	
SPECIAL TOXICITY STUDIES			10.00	16,400	
DATA PROCESSING AND SUPPORT			5.00	8,200	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
TREATMENT STUDIES		* 20.00*	32,800	
CHEMOTHERAPY				
HEALTH RESEARCH, INC.	N01CM57039 D.T.P.			104,515
BIOLOGICAL STUDIES		*100.00*	104,515	
IMMUNOBIOLOGY		40.00	41,806	
IMMUNOSUPPRESSION		60.00	62,709	
HEM RESEARCH, INC.	N01CM67066 D.T.P.			26,106
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	13,053	
VIRUS & CELLS				
BIOLOGICAL STUDIES		* 50.00*	13,053	
MARKERS		10.00	2,611	
MOLECULAR BIOLOGY		40.00	10,442	
HOUSTON, UNIVERSITY OF	N01CM87209 D.T.P.			128,101
ACQUISITION OF MATERIALS		*100.00*	128,101	
NEW AGENT PROCUREMENT, MARINE/ARTHROPODS				
IIT RESEARCH INSTITUTE	N01CM63832 D.T.P.			82,150
ACQUISITION OF MATERIALS		* 5.00*	4,108	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 95.00*	78,043	
PRIMARY SCREENING, IN VITRO		90.00	73,935	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	4,108	
IIT RESEARCH INSTITUTE	N01CM43755 D.T.P.			302,151
ACQUISITION OF MATERIALS		* 20.00*	60,430	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 65.00*	196,398	
PRIMARY SCREENING, IN VITRO		5.00	15,108	
PRIMARY SCREENING, IN VIVO		55.00	166,183	
DATA PROCESSING AND SUPPORT		5.00	15,108	
VERIFICATION SCREEN		* 15.00*	45,323	
DETAILED DRUG EVALUATION, IN VIVO				
IIT RESEARCH INSTITUTE	N01CM57004 D.T.P.			764,100
ACQUISITION OF MATERIALS		* 15.00*	114,615	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 71.00*	542,511	
PRIMARY SCREENING, IN VIVO		64.00	489,024	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		7.00	53,487	
VERIFICATION SCREEN		* 11.00*	84,051	
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES		* 3.00*	22,923	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
CHEMOTHERAPY				
ILLINOIS, UNIVERSITY OF	N01CM67090 D.T.P.			120,656
ACQUISITION OF MATERIALS		* 85.00*	102,558	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	78,426	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	18,098	
BIOASSAY OF NATURAL PRODUCTS		5.00	6,033	
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	18,098	
PLANT PRODUCTS		10.00	12,066	
FERMENTATION/ANTIBIOTICS		5.00	6,033	
INDIANA UNIVERSITY FOUNDATION	N01CM67057 CTEP			52,588
PROGRAM MANAGEMENT		* 10.00*	5,259	
ADMINISTRATION				
PHASE II CLINICAL TRIALS		* 30.00*	15,776	
CNS TUMORS RAD.				
PHASE III CLINICAL TRIALS		* 60.00*	31,553	
CNS TUMORS RAD.		30.00	15,776	
CNS TUMORS CHEM.		30.00	15,776	
INFORMATION MANAGEMENT SERVICES,	N01CP71010 CTEP			30,988
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	30,988	
LUNG DATA PROC. & SUPP.				
INFORMATION PLANNING ASSOC.	N01CM77104 CTEP			150,000
PROGRAM MANAGEMENT		*100.00*	150,000	
DATA PROCESSING AND SUPPORT				
INSTITUT JULES BORDET	N01CM57040 D.T.P.			178,860
BASIC SCREEN		* 80.00*	143,088	
PRIMARY SCREENING, IN VIVO				
VERIFICATION SCREEN		* 20.00*	35,772	
DETAILED DRUG EVALUATION, IN VIVO				
INSTITUT JULES BORDET	N01CM53840 O.D.			81,900
ACQUISITION OF MATERIALS		* 30.00*	24,570	
NEW AGENT PROCUREMENT, SYNTHETICS		25.00	20,475	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		5.00	4,095	
BIOLOGICAL STUDIES		* 20.00*	16,380	
DRUG EVAL./INVEST.				
PROGRAM MANAGEMENT		* 50.00*	40,950	
COMMUNICATION AND EDUCATION		30.00	24,570	
RESOURCE DEVELOPMENT		5.00	4,095	
DATA PROCESSING AND SUPPORT		15.00	12,285	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
INSTITUTE OF CANCER RESEARCH	N01CM77139 D.T.P.			189,000
ACQUISITION OF MATERIALS		*100.00*	189,000	
NEW AGENT PROCUREMENT, SYNTHETICS				
IOWA, UNIVERSITY OF	N01CM43743 D.T.P.			241,032
FORMULATION		* 20.00*	48,206	
DEVEL. OF EXP. FORMULATIONS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	192,826	
FORMULATION		70.00	168,722	
ANALYTICAL AND QUALITY CONTROL		10.00	24,103	
IOWA, UNIVERSITY OF	N01CM77176 D.T.P.			100,000
ACQUISITION OF MATERIALS		*100.00*	100,000	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT				
IOWA, UNIVERSITY OF	N01HB62973 O.D.			59,824
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	59,824	
OTHER TRANSFUSION RES.				
IST. NAZION PER LO STUD E LA CURA DEI	N01CM33714 CTEP			117,921
PHASE II CLINICAL TRIALS		* 15.00*	17,688	
COLORECTAL CHEM.		10.00	11,792	
STOMACH CHEM.		5.00	5,896	
PHASE III CLINICAL TRIALS		* 35.00*	41,272	
BREAST CHEM.				
PHASE IV CLINICAL TRIALS		* 50.00*	58,961	
BREAST SURG.		16.00	18,867	
BREAST RAD.		6.00	7,075	
BREAST CHEM.		16.00	18,867	
CNS TUMORS SURG.		4.00	4,717	
CNS TUMORS RAD.		4.00	4,717	
CNS TUMORS CHEM.		4.00	4,717	
IST. NAZION PER LO STUD E LA CURA DEI	N01CM43726 CTEP			104,912
PHASE II CLINICAL TRIALS		* 40.00*	41,965	
MELANOMA CHEM.				
PHASE IV CLINICAL TRIALS		* 60.00*	62,947	
MELANOMA SURG.		20.00	20,982	
MELANOMA CHEM.		20.00	20,982	
MELANOMA IMM.		20.00	20,982	
JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054 O.D.			49,000
ACQUISITION OF MATERIALS		* 10.00*	4,900	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
NEW AGENT PROCUREMENT, SYNTHETICS		5.00	2,450	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		5.00	2,450	
BASIC SCREEN		* 40.00*	19,600	
PRIMARY SCREENING, IN VITRO		2.00	980	
PRIMARY SCREENING, IN VIVO		18.00	8 820	
DRUG EVALUATION		20.00	9,800	
PROGRAM MANAGEMENT		* 20.00*	9,800	
COMMUNICATION AND EDUCATION		10.00	4,900	
RESOURCE DEVELOPMENT		10.00	4,900	
PHASE I CLINICAL TRIALS		* 15.00*	7,350	
LEUK.-ACUTE GRAN. CHEM.		5.00	2,450	
LEUK.-ACUTE LYMPH. CHEM.		5.00	2,450	
NON-SPECIFIC CHEM.		5.00	2,450	
PHASE II CLINICAL TRIALS		* 15.00*	7,350	
LEUK.-ACUTE GRAN. CHEM.		5.00	2,450	
LEUK.-ACUTE LYMPH. CHEM.		5.00	2,450	
NON-SPECIFIC CHEM.		5.00	2,450	
KANSAS, UNIVERSITY OF	N01CM23217 D.T.P.			62,265
FORMULATION DEVEL. OF EXP. FORMULATIONS		*100.00*	62,265	
KANSAS, UNIVERSITY OF-MEDICAL CENTER	N01CM43802 CTEP			27,826
PHASE I CLINICAL TRIALS NON-SPECIFIC CHEM.		*100.00*	27,826	
KANSAS, UNIVERSITY OF-MEDICAL CENTER	N01CM12184 D.T.P.			50,000
CLINICAL TRIALS SUPPORTIVE RESEARCH NON-SPECIFIC SPEC. PHARM./TOX.		*100.00*	50,000	
KENTUCKY, UNIVERSITY OF	N01CM67058 CTEP			3,884
PROGRAM MANAGEMENT ADMINISTRATION		* 10.00*	388	
PHASE II CLINICAL TRIALS CNS TUMORS RAD.		* 30.00*	1,165	
PHASE III CLINICAL TRIALS		* 60.00*	2,330	
CNS TUMORS RAD.		30.00	1,165	
CNS TUMORS CHEM.		30.00	1,165	
KENTUCKY, UNIVERSITY OF	N01CM67038 D.T.P.			159,075
CLINICAL TRIALS SUPPORTIVE RESEARCH LEUK.-ACUTE LYMPH. MARKERS		*100.00*	159,075	
LABORATORY RESEARCH ENTERPRISES, INC.	N01CM60124 A.P.			30,900
PHARMACOLOGY/TOXICOLOGY		*100.00*	30,900	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ANIMALS				
LABORATORY SUPPLY COMPANY, INC.	N01CM50577 A.P.			240,240
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	48,048	
BASIC SCREEN ANIMALS		* 55.00*	132,132	
VERIFICATION SCREEN ANIMALS		* 25.00*	60,060	
LABORATORY SUPPLY COMPANY, INC.	N01CM53775 A.P.			146,739
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	29,348	
BASIC SCREEN ANIMALS		* 55.00*	80,706	
VERIFICATION SCREEN ANIMALS		* 25.00*	36,685	
LEEDS, UNIVERSITY OF	N01CM43727 CTEP			32,900
PHASE II CLINICAL TRIALS		* 50.00*	16,450	
COLORECTAL CHEM.		38.00	12,502	
STOMACH CHEM.		12.00	3,948	
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 50.00*	16,450	
COLORECTAL MARKERS		38.00	12,502	
STOMACH MARKERS		12.00	3,948	
LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM77165 A.P.			525,189
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	105,038	
BASIC SCREEN ANIMALS		* 55.00*	288,854	
VERIFICATION SCREEN ANIMALS		* 25.00*	131,297	
LITTON BIONETICS, INC.	N01CM53778 A.P.			73,500
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	14,700	
BASIC SCREEN ANIMALS		* 55.00*	40,425	
VERIFICATION SCREEN ANIMALS		* 25.00*	18,375	
LITTON BIONETICS, INC.	N01CM67067 C.C.P.			65,498
PROD. AND FORM. FOR CLINICAL TRIALS DATA PROCESSING AND SUPPORT		* 10.00*	6,550	

NAME	# & AREA	PERCENT EFFORT		DOLLAR LEVEL
BIOLOGICAL STUDIES IMMUNOLOGY		* 50.00*	58,948	
LITTON BIONETICS, INC.	N01CM87169 C.O.P.			250,000
BIOLOGICAL STUDIES		* 70.00*	175,000	
BIOCHEMISTRY		20.00	50,000	
CELL KINETICS		20.00	50,000	
MARKERS		10.00	25,000	
NUTRITION		20.00	50,000	
TREATMENT STUDIES		* 30.00*	75,000	
SURGERY		10.00	25,000	
RADIOTHERAPY		10.00	25,000	
CHEMOTHERAPY		10.00	25,000	
LITTON BIONETICS, INC.	N01CO75380 D.T.P.			3,863,114
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		* 48.00*	1,854,295	
BASIC SCREEN		* 12.00*	463,574	
PRIMARY SCREENING, IN VITRO		3.00	115,893	
ANIMALS		9.00	347,680	
VERIFICATION SCREEN ANIMALS		* 30.00*	1,158,934	
PROCUREMENT OF PRECLINICAL MATERIAL FERMENTATION/ANTIBIOTICS		* 10.00*	386,311	
LITTON BIONETICS, INC.	N01CM12340 D.T.P.			97,286
PROCUREMENT OF PRECLINICAL MATERIAL ANTIBODIES/ANTIGENS		* 50.00*	48,643	
BIOLOGICAL STUDIES MOLECULAR BIOLOGY		* 50.00*	48,643	
LITTON BIONETICS, INC.	N01CM22062 D.T.P.			107,005
BIOLOGICAL STUDIES		*100.00*	107,005	
BLOOD PRODUCTS		40.00	42,802	
TISSUE CULTURE CELLS		60.00	64,203	
LITTON BIONETICS, INC.	N01CM77134 D.T.P.			727,142
PROCUREMENT OF PRECLINICAL MATERIAL ANTIBODIES/ANTIGENS		* 50.00*	363,571	
BIOLOGICAL STUDIES MOLECULAR BIOLOGY		* 50.00*	363,571	
MAKERERE UNIVERSITY COLLEGE COUNCIL	N01CM71343 C.O.P.			25,000
PHASE III CLINICAL TRIALS		*100.00*	25,000	
LYMPHOMA-BURKITT'S CHEM.		60.00	15,000	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
LYMPHOMA-HODGKIN'S	CHEM.		20.00	5,000	
HEPATOBILIARY	CHEM.		20.00	5,000	
MARIO NEGRI INST OF PHARMACOLOGICAL		N01CM53826 D.T.P.			96,809
TREATMENT STUDIES			*100.00*	96,809	
COMB. MODAL. THERAPY			100.00	96,809	
COMB. MODAL. THERAPY - CHEM.			40.00	38,724	
COMB. MODAL. THERAPY - IMM.			60.00	58,085	
MARIO NEGRI INST OF PHARMACOLOGICAL		N01CM33720 D.T.P.			304,430
ACQUISITION OF MATERIALS			* 15.00*	45,665	
NEW AGENT PROCUREMENT, SYNTHETICS					
BASIC SCREEN			* 70.00*	213,101	
PRIMARY SCREENING, IN VIVO					
VERIFICATION SCREEN			* 15.00*	45,665	
DETAILED DRUG EVALUATION, IN VIVO			10.00	30,443	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL			5.00	15,222	
MARIO NEGRI INST OF PHARMACOLOGICAL		N01CM67064 D.T.P.			75,580
PHARMACOLOGY/TOXICOLOGY			*100.00*	75,580	
PHARMACOLOGY					
MARSHALL RESEARCH ANIMALS, INC.		N01CM60123 A.P.			29,400
PHARMACOLOGY/TOXICOLOGY			*100.00*	29,400	
ANIMALS					
MARYLAND, UNIVERSITY OF		N01CM43748 BCRC			3,630,000
PROGRAM MANAGEMENT			* 5.00*	181,500	
ADMINISTRATION					
PHASE I CLINICAL TRIALS			* 10.00*	363,000	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 34.00*	1,234,200	
LEUK.-ACUTE GRAN.	CHEM.		3.00	108,900	
LEUK.-ACUTE GRAN.	SUPPORTIVE CARE		1.00	36,300	
LEUK.-ACUTE LYMPH.	CHEM.		1.00	36,300	
LEUK.-CHRON. GRAN.	CHEM.		1.00	36,300	
LEUK.-CHRON. GRAN.	SUPPORTIVE CARE		1.00	36,300	
LEUK.-CHRON LYMPH.	CHEM.		1.00	36,300	
LYMPHOMA-HODGKIN'S	CHEM.		1.00	36,300	
LYMPHOMA-HODGKIN'S	SUPPORTIVE CARE		1.00	36,300	
LYMPHOMA-LYMPHOCYT	CHEM.		1.00	36,300	
BREAST	CHEM.		3.00	108,900	
BREAST	SUPPORTIVE CARE		1.00	36,300	
CNS TUMORS	RAD.		1.00	36,300	
CNS TUMORS	CHEM.		3.00	108,900	
CNS TUMORS	SUPPORTIVE CARE		2.00	72,600	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
COLORECTAL	CHEM.		2.00	72,600	
COLORECTAL	SUPPORTIVE CARE		1.00	36,300	
KIDNEY	CHEM.		1.00	36,300	
MELANOMA	CHEM.		2.00	72,600	
OSTEOGENIC	CHEM.		3.00	108,900	
OSTEOGENIC	SUPPORTIVE CARE		1.00	36,300	
NON-SPECIFIC	RAD.		2.00	72,600	
NON-SPECIFIC	CHEM.		1.00	36,300	
PHASE III CLINICAL TRIALS			* 26.00*	943,800	
LEUK.-ACUTE GRAN.	CHEM.		3.00	108,900	
LEUK.-ACUTE GRAN.	SUPPORTIVE CARE		1.00	36,300	
LEUK.-ACUTE LYMPH.	CHEM.		1.00	36,300	
LEUK.-ACUTE LYMPH.	SUPPORTIVE CARE		1.00	36,300	
LEUK.-CHRON. GRAN.	CHEM.		1.00	36,300	
LYMPHOMA-HODGKIN'S	CHEM.		3.00	108,900	
LYMPHOMA-HODGKIN'S	SUPPORTIVE CARE		2.00	72,600	
LYMPHOMA-LYMPHOCYT	CHEM.		2.00	72,600	
LYMPHOMA-MYCO.FUNG	CHEM.		2.00	72,600	
MYELOMA	CHEM.		2.00	72,600	
MYELOMA	SUPPORTIVE CARE		1.00	36,300	
LUNG	CHEM.		3.00	108,900	
TESTICULAR	CHEM.		2.00	72,600	
NON-SPECIFIC	CHEM.		1.00	36,300	
NON-SPECIFIC	SUPPORTIVE CARE		1.00	36,300	
PHASE IV CLINICAL TRIALS			* 25.00*	907,500	
LEUK.-ACUTE GRAN.	CHEM.		5.00	181,500	
LEUK.-ACUTE GRAN.	SUPPORTIVE CARE		2.00	72,600	
LEUK.-ACUTE LYMPH.	CHEM.		1.00	36,300	
LEUK.-CHRON. GRAN.	CHEM.		1.00	36,300	
LEUK.-CHRON LYMPH.	CHEM.		1.00	36,300	
LEUK.-CHRON LYMPH.	SUPPORTIVE CARE		1.00	36,300	
LYMPHOMA-HODGKIN'S	SURG.		3.00	108,900	
LYMPHOMA-HODGKIN'S	RAD.		3.00	108,900	
LYMPHOMA-HODGKIN'S	CHEM.		3.00	108,900	
LYMPHOMA-HODGKIN'S	SUPPORTIVE CARE		3.00	108,900	
LYMPHOMA-LYMPHOCYT	CHEM.		1.00	36,300	
NON-SPECIFIC	CHEM.		1.00	36,300	
MARYLAND, UNIVERSITY OF		N01CM87223 CTEP			83,740
PHASE IV CLINICAL TRIALS			*100.00*	83,740	
HEAD AND NECK	SURG.		30.00	25,122	
HEAD AND NECK	RAD.		30.00	25,122	
HEAD AND NECK	CHEM.		20.00	16,748	
HEAD AND NECK	SUPPORTIVE CARE		20.00	16,748	
MASON RESEARCH INSTITUTE		N01CM87164 A.P.			125,430
BASIC SCREEN			* 8.00*	10,034	
TUMOR BANK					
PHARMACOLOGY/TOXICOLOGY			* 77.00*	96,581	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PHARMACOLOGY				
BIOLOGICAL STUDIES		* 15.00*	18,815	
BIOCHEMISTRY				
MASON RESEARCH INSTITUTE	N01CM67011 C.O.P.			209,262
TREATMENT STUDIES		*100.00*	209,262	
DATA PROCESSING AND SUPPORT				
MASON RESEARCH INSTITUTE	N01CM57003 D.T.P.			678,023
ACQUISITION OF MATERIALS		* 5.00*	33,901	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 61.00*	413,594	
PRIMARY SCREENING, IN VIVO		50.00	339,012	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		11.00	74,583	
VERIFICATION SCREEN		* 24.00*	162,726	
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES		* 10.00*	67,802	
COMB. MODAL. THERAPY		10.00	67,802	
COMB. MODAL. THERAPY - SURG.		8.00	54,242	
COMB. MODAL. THERAPY - CHEM.		2.00	13,560	
MASON RESEARCH INSTITUTE	N01CM57030 D.T.P.			173,700
VERIFICATION SCREEN		*100.00*	173,700	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL				
MAYO FOUNDATION	N01CM53772 CTEP			189,446
PHASE II CLINICAL TRIALS		*100.00*	189,446	
BREAST CHEM.				
MAYO FOUNDATION	N01CM57033 CTEP			152,225
PHASE IV CLINICAL TRIALS		* 80.00*	121,780	
COLORECTAL SURG.		10.00	15,223	
COLORECTAL RAD.		30.00	45,668	
COLORECTAL CHEM.		40.00	60,890	
OTHER CLINICAL TRIALS RESEARCH		* 20.00*	30,445	
COLORECTAL DATA PROC. & SUPP.				
MAYO FOUNDATION	N01CM77150 CTEP			46,394
PHASE II CLINICAL TRIALS		* 20.00*	9,279	
LUNG RAD.		10.00	4,639	
LUNG CHEM.		10.00	4,639	
PHASE III CLINICAL TRIALS		* 60.00*	27,836	
LUNG RAD.		30.00	13,918	
LUNG CHEM.		30.00	13,918	
PHASE IV CLINICAL TRIALS		* 20.00*	9,279	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
LUNG	RAD.		10.00	4,639	
LUNG	CHEM.		10.00	4,639	
MAYO FOUNDATION		N01CM57044 CTEP			556,575
PHASE II CLINICAL TRIALS			* 55.00*	306,116	
BREAST	CHEM.		15.00	83,486	
COLORECTAL	CHEM.		15.00	83,486	
HEAD AND NECK	CHEM.		5.00	27,829	
LUNG	CHEM.		5.00	27,829	
MELANOMA	CHEM.		10.00	55,658	
SARCOMAS (GEN.)	CHEM.		5.00	27,829	
PHASE III CLINICAL TRIALS			* 45.00*	250,459	
BREAST	CHEM.		10.00	55,658	
COLORECTAL	CHEM.		5.00	27,829	
HEAD AND NECK	CHEM.		5.00	27,829	
LUNG	CHEM.		5.00	27,829	
MELANOMA	CHEM.		15.00	83,486	
SARCOMAS (GEN.)	CHEM.		5.00	27,829	
MAYO FOUNDATION		N01CM53838 CTEP			49,571
PHASE I CLINICAL TRIALS			* 80.00*	39,657	
NON-SPECIFIC	CHEM.		50.00	24,786	
NON-SPECIFIC	PHARM./TOX.		30.00	14,871	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	9,914	
NON-SPECIFIC	PROTOCOL-REL. STAT		5.00	2,479	
NON-SPECIFIC	DATA PROC. & SUPP.		15.00	7,436	
MAYO FOUNDATION		N01CM43783 CTEP			124,000
PHASE III CLINICAL TRIALS			* 40.00*	49,600	
STOMACH	CHEM.				
PHASE IV CLINICAL TRIALS			* 45.00*	55,800	
STOMACH	SURG.		10.00	12,400	
STOMACH	RAD.		10.00	12,400	
STOMACH	CHEM.		25.00	31,000	
OTHER CLINICAL TRIALS RESEARCH			* 15.00*	18,600	
STOMACH	DATA PROC. & SUPP.				
MAYO FOUNDATION		N01CM43796 CTEP			181,000
PHASE II CLINICAL TRIALS			* 30.00*	54,300	
PANCREATIC	CHEM.				
PHASE IV CLINICAL TRIALS			* 50.00*	90,500	
PANCREATIC	SURG.		5.00	9,050	
PANCREATIC	RAD.		20.00	36,200	
PANCREATIC	CHEM.		25.00	45,250	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	36,200	
PANCREATIC	DATA PROC. & SUPP.				

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
MAYO FOUNDATION		N01CM02066 CTEP			30,625
PHASE II CLINICAL TRIALS			* 75.00*	22,969	
COLORECTAL	CHEM.		60.00	18,375	
PANCREATIC	CHEM.		7.00	2,144	
STOMACH	CHEM.		8.00	2,450	
PHASE III CLINICAL TRIALS			* 25.00*	7,656	
COLORECTAL	CHEM.		20.00	6,125	
PANCREATIC	CHEM.		3.00	919	
STOMACH	CHEM.		2.00	613	
MAYO FOUNDATION		N01CM67123 CTEP			224,975
PHASE III CLINICAL TRIALS			* 50.00*	112,488	
OVARIAN	SURG.		15.00	33,746	
OVARIAN	RAD.		15.00	33,746	
OVARIAN	CHEM.		20.00	44,995	
PHASE IV CLINICAL TRIALS			* 50.00*	112,488	
OVARIAN	SURG.		15.00	33,746	
OVARIAN	RAD.		15.00	33,746	
OVARIAN	CHEM.		20.00	44,995	
MEDICAL RESEARCH COUNCIL		N01CM87171 D.T.P.			40,953
FORMULATION			* 20.00*	8,191	
LIPOSOME ENCAPS.					
VERIFICATION SCREEN			* 30.00*	12,286	
DETAILED DRUG EVALUATION, IN VITRO			10.00	4,095	
DETAILED DRUG EVALUATION, IN VIVO			15.00	6,143	
DATA PROCESSING AND SUPPORT			5.00	2,048	
PHARMACOLOGY/TOXICOLOGY			* 30.00*	12,286	
PHARMACOLOGY			15.00	6,143	
SPECIAL TOXICITY STUDIES			10.00	4,095	
DATA PROCESSING AND SUPPORT			5.00	2,048	
TREATMENT STUDIES			* 20.00*	8,191	
CHEMOTHERAPY					
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM87224 CTEP			81,662
PHASE IV CLINICAL TRIALS			*100.00*	81,662	
HEAD AND NECK	SURG.		30.00	24,499	
HEAD AND NECK	RAD.		30.00	24,499	
HEAD AND NECK	CHEM.		20.00	16,332	
HEAD AND NECK	SUPPORTIVE CARE		20.00	16,332	
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM53844 CTEP			66,980
PHASE II CLINICAL TRIALS			* 16.00*	10,717	
COLORECTAL	RAD.		3.00	2,009	
COLORECTAL	CHEM.		10.00	6,698	

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
COLORECTAL	IMM.		3.00	2,009
PHASE III CLINICAL TRIALS			* 84.00*	56,263
COLORECTAL	RAD.		17.00	11,387
COLORECTAL	CHEM.		50.00	33,490
COLORECTAL	IMM.		17.00	11,387
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM57043 CTEP		381,106
PHASE II CLINICAL TRIALS			* 60.00*	228,664
BREAST	CHEM.		5.00	19,055
CNS TUMORS	CHEM.		5.00	19,055
HEAD AND NECK	CHEM.		5.00	19,055
LUNG	CHEM.		20.00	76,221
MELANOMA	CHEM.		5.00	19,055
SARCOMAS (GEN.)	CHEM.		10.00	38,111
STOMACH	CHEM.		5.00	19,055
TESTICULAR	CHEM.		5.00	19,055
PHASE III CLINICAL TRIALS			* 40.00*	152,442
BREAST	CHEM.		5.00	19,055
COLORECTAL	CHEM.		5.00	19,055
HEAD AND NECK	CHEM.		5.00	19,055
LUNG	CHEM.		5.00	19,055
MELANOMA	CHEM.		5.00	19,055
STOMACH	CHEM.		5.00	19,055
TESTICULAR	CHEM.		10.00	38,111
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM57019 CTEP		149,472
PROGRAM MANAGEMENT ADMINISTRATION			* 10.00*	14,947
PHASE II CLINICAL TRIALS			* 30.00*	44,842
CNS TUMORS	RAD.			
PHASE III CLINICAL TRIALS			* 60.00*	89,683
CNS TUMORS	RAD.		30.00	44,842
CNS TUMORS	CHEM.		30.00	44,842
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM43804 CTEP		29,712
PHASE I CLINICAL TRIALS			*100.00*	29,712
NON-SPECIFIC	CHEM.			
MIAMI, UNIVERSITY OF		N01CM43800 CTEP		183,741
PHASE II CLINICAL TRIALS			* 35.00*	64,309
PANCREATIC	SURG.		1.75	3,215
PANCREATIC	CHEM.		21.00	38,586
PANCREATIC	IMM.		12.25	22,508
PHASE III CLINICAL TRIALS			* 55.00*	101,058
PANCREATIC	SURG.		5.50	10,106
PANCREATIC	RAD.		19.25	35,370
PANCREATIC	CHEM.		19.25	35,370

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PANCREATIC	IMM.		11.00	20,212	
PHASE IV CLINICAL TRIALS			* 10.00*	18,374	
PANCREATIC	SURG.		1.00	1,837	
PANCREATIC	RAD.		2.00	3,675	
PANCREATIC	CHEM.		5.00	9,187	
PANCREATIC	IMM.		2.00	3,675	
MIAMI, UNIVERSITY OF		N01CM67093 CTEP			106,843
PHASE IV CLINICAL TRIALS			*100.00*	106,843	
COLORECTAL	SURG.		20.00	21,369	
COLORECTAL	RAD.		15.00	16,026	
COLORECTAL	CHEM.		45.00	48,079	
COLORECTAL	IMM.		20.00	21,369	
MIAMI, UNIVERSITY OF		N01CM57041 D.T.P.			26,540
BIOLOGICAL STUDIES			*100.00*	26,540	
IMMUNOBIOLOGY			40.00	10,616	
IMMUNOSUPPRESSION			60.00	15,924	
MIAMI, UNIVERSITY OF		N01CM67077 D.T.P.			137,508
ACQUISITION OF MATERIALS			* 5.00*	6,875	
BIOASSAY OF NATURAL PRODUCTS					
BASIC SCREEN			* 95.00*	130,633	
PRIMARY SCREENING, IN VITRO			90.00	123,757	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			5.00	6,875	
MICHIGAN DEPARTMENT OF PUBLIC HEALTH		N01CM33702 D.T.P.			64,000
BASIC SCREEN			*100.00*	64,000	
PRIMARY SCREENING, IN VITRO					
MICHIGAN, UNIVERSITY OF		N01CM87225 CTEP			133,680
PHASE IV CLINICAL TRIALS			*100.00*	133,680	
HEAD AND NECK	SURG.		30.00	40,104	
HEAD AND NECK	RAD.		30.00	40,104	
HEAD AND NECK	CHEM.		20.00	26,736	
HEAD AND NECK	SUPPORTIVE CARE		20.00	26,736	
MICROBIAL CHEMISTRY RESEARCH FDN.		N01CM57009 D.T.P.			150,000
ACQUISITION OF MATERIALS			* 60.00*	90,000	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT					
BASIC SCREEN			* 40.00*	60,000	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL					

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
MICROBIOLOGICAL ASSOCIATES	N01CM67069 A.P.			200,150
ACQUISITION OF MATERIALS		* 20.00*	40,030	
ANIMALS				
BASIC SCREEN		* 55.00*	110,083	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	50,038	
ANIMALS				
MICROBIOLOGICAL ASSOCIATES	N01CM53818 A.P.			70,000
ACQUISITION OF MATERIALS		* 20.00*	14,000	
ANIMALS				
BASIC SCREEN		* 55.00*	38,500	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	17,500	
ANIMALS				
MICROBIOLOGICAL ASSOCIATES	N01CM77131 BCRC			85,700
PROGRAM MANAGEMENT		* 7.00*	5,999	
ADMINISTRATION		2.00	1,714	
DATA PROCESSING AND SUPPORT		5.00	4,285	
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 93.00*	79,701	
LEUK.-ACUTE GRAN. VIROLOGY		30.00	25,710	
LEUK.-ACUTE LYMPH. VIROLOGY		5.00	4,285	
LEUK.-CHRON. GRAN. VIROLOGY		10.00	8,570	
LEUK.-CHRON LYMPH. VIROLOGY		5.00	4,285	
LYMPHOMA-HODGKIN'S VIROLOGY		20.00	17,140	
LYMPHOMA-LYMPHOCYT VIROLOGY		5.00	4,285	
LYMPHOMA-MYCO.FUNG VIROLOGY		3.00	2,571	
MYELOMA VIROLOGY		5.00	4,285	
CNS TUMORS VIROLOGY		5.00	4,285	
MELANOMA VIROLOGY		5.00	4,285	
MICROBIOLOGICAL ASSOCIATES	N01CM63810 C.O.P.			50,000
BIOLOGICAL STUDIES		*100.00*	50,000	
IMMUNOBIOLOGY		50.00	25,000	
SERUM STORAGE		50.00	25,000	
MICROBIOLOGICAL ASSOCIATES	N01CM43793 D.T.P.			425,000
ACQUISITION OF MATERIALS		* 60.00*	255,000	
DATA PROCESSING AND SUPPORT		10.00	42,500	
STORAGE DISTRIBUTION		50.00	212,500	
PROCUREMENT OF PRECLINICAL MATERIAL		* 30.00*	127,500	
STORAGE DISTRIBUTION				
PROD. AND FORM. FOR CLINICAL TRIALS		* 10.00*	42,500	
DATA PROCESSING AND SUPPORT		1.00	4,250	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
STORAGE DISTRIBUTION		9.00	38,250	
MIDWEST RESEARCH INSTITUTE	N01CM33722 D.T.P.			52,700
PROCUREMENT OF PRECLINICAL MATERIAL ANALYTICAL AND QUALITY CONTROL		* 20.00*	10,540	
PROD. AND FORM. FOR CLINICAL TRIALS ANALYTICAL AND QUALITY CONTROL		* 80.00*	42,160	
MINNESOTA, UNIVERSITY OF	N01HB62972 O.D.			59,032
CLINICAL TRIALS SUPPORTIVE RESEARCH OTHER TRANSFUSION RES.		*100.00*	59,032	
MISSOURI, UNIVERSITY OF	N01CM87157 A.P.			76,015
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	15,203	
BASIC SCREEN ANIMALS		* 55.00*	41,808	
VERIFICATION SCREEN ANIMALS		* 25.00*	19,004	
MISSOURI, UNIVERSITY OF	N01CM12323 D.T.P.			55,000
CLINICAL TRIALS SUPPORTIVE RESEARCH NON-SPECIFIC MARKERS		*100.00*	55,000	
MONSANTO RESEARCH CORPORATION	N01CM67046 D.T.P.			1,101,994
PROCUREMENT OF PRECLINICAL MATERIAL PLANT PRODUCTS		* 10.00*	110,199	
PROD. AND FORM. FOR CLINICAL TRIALS		* 90.00*	991,795	
PRODUCTION, SYNTHETICS		15.00	165,299	
PRODUCTION, PLANT PRODUCTS		75.00	826,496	
MONTEFIORE HOSPITAL	N01CM67061 CTEP			103,504
PROGRAM MANAGEMENT ADMINISTRATION		* 10.00*	10,350	
PHASE II CLINICAL TRIALS CNS TUMORS RAD.		* 30.00*	31,051	
PHASE III CLINICAL TRIALS		* 60.00*	62,102	
CNS TUMORS RAD.		30.00	31,051	
CNS TUMORS CHEM.		30.00	31,051	
MOUNT SINAI SCHOOL OF MEDICINE	N01CM53841 CTEP			184,921
PHASE IV CLINICAL TRIALS		* 90.00*	166,429	
STOMACH SURG.		25.00	46,230	
STOMACH RAD.		25.00	46,230	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
STOMACH	CHEM.		40.00	73,968	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	18,492	
STOMACH	MARKERS				
OUNT SINAI SCHOOL OF MEDICINE		N01CM53837 CTEP			89,997
PHASE I CLINICAL TRIALS			* 80.00*	71,998	
NON-SPECIFIC	CHEM.		50.00	44,999	
NON-SPECIFIC	PHARM./TOX.		30.00	26,999	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	17,999	
NON-SPECIFIC	PROTOCOL-REL. STAT		5.00	4,500	
NON-SPECIFIC	DATA PROC. & SUPP.		15.00	13,500	
MOUNT SINAI SCHOOL OF MEDICINE		N01CM67096 CTEP			135,246
PHASE IV CLINICAL TRIALS			* 90.00*	121,721	
COLORECTAL	SURG.		18.00	24,344	
COLORECTAL	RAD.		18.00	24,344	
COLORECTAL	CHEM.		36.00	48,689	
COLORECTAL	IMM.		18.00	24,344	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	13,525	
COLORECTAL	MARKERS				
MURPHY BREEDING LABS., INC.		N01CM50579 A.P.			224,640
ACQUISITION OF MATERIALS			* 20.00*	44,928	
ANIMALS					
BASIC SCREEN			* 55.00*	123,552	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	56,160	
ANIMALS					
NATIONAL ACADEMY OF SCIENCES		N01CM57013 A.P.			25,000
PROGRAM MANAGEMENT			*100.00*	25,000	
COMMUNICATION AND EDUCATION					
NATIONAL ACADEMY OF SCIENCES		N01CM53850 A.P.			20,000
PROGRAM MANAGEMENT			*100.00*	20,000	
COMMUNICATION AND EDUCATION					
NATIONAL NAVAL MEDICAL CENTER		Y01CM50100 CTEP			38,100
PHASE III CLINICAL TRIALS			*100.00*	38,100	
BREAST	CHEM.				
NEW YORK UNIVERSITY MEDICAL CENTER		N01CM67059 CTEP			121,561
PROGRAM MANAGEMENT			* 10.00*	12,156	
ADMINISTRATION					

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PHASE II CLINICAL TRIALS			* 30.00*	36,468	
CNS TUMORS	CHEM.				
PHASE III CLINICAL TRIALS			* 60.00*	72,937	
CNS TUMORS	RAD.		30.00	36,468	
CNS TUMORS	CHEM.		30.00	36,468	
NORTHERN CALIFORNIA CANCER PROGRAM		N01CM87154 CTEP			177,814
PHASE IV CLINICAL TRIALS			*100.00*	177,814	
HEAD AND NECK	SURG.		30.00	53,344	
HEAD AND NECK	RAD.		30.00	53,344	
HEAD AND NECK	CHEM.		20.00	35,563	
HEAD AND NECK	SUPPORTIVE CARE		20.00	35,563	
OHIO STATE UNIVERSITY RESEARCH		N01CM67060 CTEP			48,488
PROGRAM MANAGEMENT			* 10.00*	4,849	
ADMINISTRATION					
PHASE II CLINICAL TRIALS			* 30.00*	14,546	
CNS TUMORS	CHEM.				
PHASE III CLINICAL TRIALS			* 60.00*	29,093	
CNS TUMORS	RAD.		30.00	14,546	
CNS TUMORS	CHEM.		30.00	14,546	
OHIO STATE UNIVERSITY RESEARCH		N01CM87161 D.T.P.			102,158
PHARMACOLOGY/TOXICOLOGY			* 25.00*	25,540	
ANAL. METH.					
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 75.00*	76,619	
NON-SPECIFIC	SPEC. PHARM./TOX.				
OHIO STATE UNIVERSITY RESEARCH		N01CM53828 D.T.P.			52,264
PHARMACOLOGY/TOXICOLOGY			* 10.00*	5,226	
PHARMACOLOGY					
PHASE I CLINICAL TRIALS			* 80.00*	41,811	
NON-SPECIFIC	PHARM./TOX.				
PHASE II CLINICAL TRIALS			* 10.00*	5,226	
NON-SPECIFIC	PHARM./TOX.				
ONTARIO CANCER INSTITUTE		N01CM77152 CTEP			188,757
PHASE II CLINICAL TRIALS			* 20.00*	37,751	
LUNG	RAD.		10.00	18,876	
LUNG	CHEM.		10.00	18,876	
PHASE III CLINICAL TRIALS			* 60.00*	113,254	
LUNG	RAD.		30.00	56,627	
LUNG	CHEM.		30.00	56,627	
PHASE IV CLINICAL TRIALS			* 20.00*	37,751	
LUNG	RAD.		10.00	18,876	
LUNG	CHEM.		10.00	18,876	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
OREGON STATE UNIVERSITY	N01CM23201 D.T.P.			46,520
PHARMACOLOGY/TOXICOLOGY PHARMACOLOGY		*100.00*	46,520	
PARKE, DAVIS AND COMPANY	N01CM87124 D.T.P.			339,905
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 50.00*	169,953	
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	169,953	
PARKE, DAVIS AND COMPANY	N01CM77146 D.T.P.			665,000
ACQUISITION OF MATERIALS		* 85.00*	565,250	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	551,950	
DATA PROCESSING AND SUPPORT		2.00	13,300	
BASIC SCREEN		* 15.00*	99,750	
PRIMARY SCREENING, IN VITRO		10.00	66,500	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	33,250	
PFIZER, INC.	N01CM53805 D.T.P.			289,000
ACQUISITION OF MATERIALS ANIMAL VIRUSES		*100.00*	289,000	
PHARM-ECO	N01CM87200 D.T.P.			258,223
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 50.00*	129,112	
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	129,112	
PHILIPS ROXANE LABORATORIES, INC.	N01CM67053 D.T.P.			134,594
FORMULATION DEVEL. OF EXP. FORMULATIONS		* 20.00*	26,919	
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	107,675	
FORMULATION		70.00	94,216	
ANALYTICAL AND QUALITY CONTROL		10.00	13,459	
PITTSBURGH, UNIVERSITY OF	N01CM77177 CTEP			716,506
PHASE IV CLINICAL TRIALS		*100.00*	716,506	
COLORECTAL SURG.		40.00	286,602	
COLORECTAL RAD.		10.00	71,651	
COLORECTAL CHEM.		50.00	358,253	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PITTSBURGH, UNIVERSITY OF		N01CB23876 CTEP			830,725
PHASE III CLINICAL TRIALS			*100.00*	830,725	
BREAST	SURG.		20.00	166,145	
BREAST	RAD.		20.00	166,145	
BREAST	CHEM.		20.00	166,145	
BREAST	IMM.		20.00	166,145	
COLORECTAL	SURG.		5.00	41,536	
COLORECTAL	RAD.		5.00	41,536	
COLORECTAL	CHEM.		5.00	41,536	
COLORECTAL	IMM.		5.00	41,536	
POLYSCIENCES, INC.		N01CM77070 D.T.P.			283,064
ACQUISITION OF MATERIALS			*100.00*	283,064	
NEW AGENT PROCUREMENT, PLANT PRODUCTS			79.00	223,621	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			10.00	28,306	
BIOASSAY OF NATURAL PRODUCTS			11.00	31,137	
POMONA COLLEGE		N01CM67062 D.T.P.			52,678
ACQUISITION OF MATERIALS			*100.00*	52,678	
STRUCTURE ACTIVITY					
PUBLIC HEALTH SERVICE		Y01CM40003 BCRC			187,225
PHARMACOLOGY/TOXICOLOGY			* 35.00*	65,529	
PHARMACOLOGY			30.00	56,168	
DATA PROCESSING AND SUPPORT			5.00	9,361	
BIOLOGICAL STUDIES			* 55.00*	102,974	
BIOCHEMISTRY			10.00	18,723	
CELL BIOLOGY			10.00	18,723	
CELL KINETICS			5.00	9,361	
IMMUNOBIOLOGY			10.00	18,723	
MARKERS			5.00	9,361	
MOLECULAR BIOLOGY			15.00	28,084	
PROGRAM MANAGEMENT			* 10.00*	18,723	
ADMINISTRATION					
PUBLIC HEALTH SERVICE		Y01CM77132 BCRC			350,000
PROGRAM MANAGEMENT			* 5.00*	17,500	
DATA PROCESSING AND SUPPORT					
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 95.00*	332,500	
LEUK.-ACUTE GRAN.	DATA PROC. & SUPP.		11.00	38,500	
LEUK.-ACUTE LYMPH.	DATA PROC. & SUPP.		4.00	14,000	
LEUK.-CHRON. GRAN.	DATA PROC. & SUPP.		3.00	10,500	
LEUK.-CHRON LYMPH.	DATA PROC. & SUPP.		1.00	3,500	
LYMPHOMA-HISTIOCYT	DATA PROC. & SUPP.		8.00	28,000	
LYMPHOMA-HODGKIN'S	DATA PROC. & SUPP.		10.00	35,000	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
LYMPHOMA-LYMPHOCYT	DATA PROC. & SUPP.	5.00	17,500	
LYMPHOMA-MYCO.FUNG	DATA PROC. & SUPP.	7.00	24,500	
MYELOMA	DATA PROC. & SUPP.	1.00	3,500	
BREAST	DATA PROC. & SUPP.	3.00	10,500	
CNS TUMORS	DATA PROC. & SUPP.	12.00	42,000	
COLORECTAL	DATA PROC. & SUPP.	2.00	7,000	
KIDNEY	DATA PROC. & SUPP.	6.00	21,000	
LUNG	DATA PROC. & SUPP.	4.00	14,000	
MELANOMA	DATA PROC. & SUPP.	5.00	17,500	
OSTEOGENIC	DATA PROC. & SUPP.	1.00	3,500	
OVARIAN	DATA PROC. & SUPP.	1.00	3,500	
RHABDOMYOSARCOMA	DATA PROC. & SUPP.	1.00	3,500	
SARCOMAS (GEN.)	DATA PROC. & SUPP.	2.00	7,000	
STOMACH	DATA PROC. & SUPP.	1.00	3,500	
TESTICULAR	DATA PROC. & SUPP.	2.00	7,000	
NON-SPECIFIC	DATA PROC. & SUPP.	5.00	17,500	
PURDUE UNIVERSITY	N01CM67091 D.T.P.			125,773
ACQUISITION OF MATERIALS		* 85.00*	106,907	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	81,752	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	18,866	
BIOASSAY OF NATURAL PRODUCTS		5.00	6,289	
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	18,866	
PLANT PRODUCTS		10.00	12,577	
FERMENTATION/ANTIBIOTICS		5.00	6,289	
RADIOBIOLOGICAL INST., ORGAN. FOR	N01CM53763 C.D.			73,151
TREATMENT STUDIES		*100.00*	73,151	
COMB. MODAL. THERAPY		100.00	73,151	
COMB. MODAL. THERAPY - RAD.		50.00	36,576	
COMB. MODAL. THERAPY - CHEM.		50.00	36,576	
RES. FDN. OF STATE UNIVERSITY OF NEW	N01CM53766 D.T.P.			151,164
BIOLOGICAL STUDIES		*100.00*	151,164	
IMMUNOBIOLOGY		40.00	60,466	
IMMUNOSUPPRESSION		60.00	90,698	
RESEARCH TRIANGLE INSTITUTE	N01CM67089 D.T.P.			194,010
ACQUISITION OF MATERIALS		* 85.00*	164,909	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	126,107	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	29,102	
BIOASSAY OF NATURAL PRODUCTS		5.00	9,701	
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	29,102	
PLANT PRODUCTS		10.00	19,401	
FERMENTATION/ANTIBIOTICS		5.00	9,701	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
RESEARCH TRIANGLE INSTITUTE		N01CM67121 D.T.P.			160,000
PROCUREMENT OF PRECLINICAL MATERIAL			* 80.00*	128,000	
RADIOLABELED MATERIALS					
PROD. AND FORM. FOR CLINICAL TRIALS			* 20.00*	32,000	
RADIOLABEL					
ROCHESTER, UNIVERSITY OF		N01CM77159 O.D.			96,000
PROGRAM MANAGEMENT			*100.00*	96,000	
PROGRAM PLANNING					
SAINT JUDE CHILDREN'S RESEARCH		N01CM77127 D.T.P.			94,000
ACQUISITION OF MATERIALS			*100.00*	94,000	
NEW AGENT PROCUREMENT, SYNTHETICS					
SAINT LOUIS UNIVERSITY SCHOOL OF		N01CM57020 CTEP			133,130
PROGRAM MANAGEMENT			* 10.00*	13,313	
ADMINISTRATION					
PHASE II CLINICAL TRIALS			* 30.00*	39,939	
CNS TUMORS	CHEM.				
PHASE III CLINICAL TRIALS			* 60.00*	79,878	
CNS TUMORS	RAD.		30.00	39,939	
CNS TUMORS	CHEM.		30.00	39,939	
SIDNEY FARBER CANCER INSTITUTE		N01CM43781 CTEP			55,846
PHASE IV CLINICAL TRIALS			*100.00*	55,846	
STOMACH	RAD.		15.00	8,377	
STOMACH	CHEM.		78.00	43,560	
STOMACH	SUPPORTIVE CARE		7.00	3,909	
SIDNEY FARBER CANCER INSTITUTE		N01CM67037 CTEP			345,745
PHASE II CLINICAL TRIALS			* 55.00*	190,160	
BREAST	CHEM.		15.00	51,862	
COLORECTAL	CHEM.		15.00	51,862	
HEAD AND NECK	CHEM.		5.00	17,287	
LUNG	CHEM.		5.00	17,287	
MELANOMA	CHEM.		10.00	34,575	
SARCOMAS (GEN.)	CHEM.		5.00	17,287	
PHASE III CLINICAL TRIALS			* 45.00*	155,585	
BREAST	CHEM.		10.00	34,575	
COLORECTAL	CHEM.		5.00	17,287	
HEAD AND NECK	CHEM.		5.00	17,287	
LUNG	CHEM.		5.00	17,287	
MELANOMA	CHEM.		15.00	51,862	
SARCOMAS (GEN.)	CHEM.		5.00	17,287	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
SIMONSEN LABORATORIES	N01CM53847 A.P.			250,003
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	50,001	
BASIC SCREEN ANIMALS		* 55.00*	137,502	
VERIFICATION SCREEN ANIMALS		* 25.00*	62,501	
SIMONSEN LABORATORIES	N01CM50578 A.P.			240,240
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	48,048	
BASIC SCREEN ANIMALS		* 55.00*	132,132	
VERIFICATION SCREEN ANIMALS		* 25.00*	60,060	
SIMONSEN LABORATORIES	N01CM77166 A.P.			11,336
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	2,267	
BASIC SCREEN ANIMALS		* 55.00*	6,235	
VERIFICATION SCREEN ANIMALS		* 25.00*	2,834	
SLOAN-KETTERING INSTITUTE FOR CANCER	N01CM53820 D.T.P.			72,366
PROCUREMENT OF PRECLINICAL MATERIAL RADIOLABELED MATERIALS		* 25.00*	18,092	
BIOLOGICAL STUDIES MOLECULAR BIOLOGY		* 75.00*	54,275	
SMALL BUSINESS ADMINISTRATION	N01CM43719 D.T.P.			159,895
ACQUISITION OF MATERIALS ANIMAL CELLS		* 70.00*	111,927	
BIOLOGICAL STUDIES		* 30.00*	47,969	
MOLECULAR BIOLOGY		10.00	15,990	
RADIOIMMUNE ASSAY		20.00	31,979	
SOUTH FLORIDA, UNIVERSITY OF	N01CM87220 CTEP			70,002
PHASE IV CLINICAL TRIALS		*100.00*	70,002	
HEAD AND NECK SURG.		30.00	21,001	
HEAD AND NECK RAD.		30.00	21,001	
HEAD AND NECK CHEM.		20.00	14,000	
HEAD AND NECK SUPPORTIVE CARE		20.00	14,000	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
SOUTHERN ANIMAL FARMS		N01CM53845 A.P.			147,630
ACQUISITION OF MATERIALS			* 20.00*	29,526	
ANIMALS					
BASIC SCREEN			* 55.00*	81,197	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	36,908	
ANIMALS					
SOUTHERN ANIMAL FARMS		N01CM50599 A.P.			229,320
ACQUISITION OF MATERIALS			* 20.00*	45,864	
ANIMALS					
BASIC SCREEN			* 55.00*	126,126	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	57,330	
ANIMALS					
SOUTHERN CALIFORNIA, UNIVERSITY OF		N01CM53842 CTEP			37,000
PHASE IV CLINICAL TRIALS			* 90.00*	33,300	
STOMACH	SURG.		25.00	9,250	
STOMACH	RAD.		25.00	9,250	
STOMACH	CHEM.		40.00	14,800	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	3,700	
STOMACH	MARKERS				
SOUTHERN CALIFORNIA, UNIVERSITY OF		N01CM67084 D.T.P.			138,757
ACQUISITION OF MATERIALS			*100.00*	138,757	
NEW AGENT PROCUREMENT, SYNTHETICS					
SOUTHERN CALIFORNIA, UNIVERSITY OF		N01CM67065 D.T.P.			127,495
PHARMACOLOGY/TOXICOLOGY			* 75.00*	95,621	
PHARMACOLOGY			65.00	82,872	
DATA PROCESSING AND SUPPORT			10.00	12,750	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 25.00*	31,874	
NON-SPECIFIC	SPEC. PHARM./TOX.		20.00	25,499	
NON-SPECIFIC	DATA PROC. & SUPP.		5.00	6,375	
SOUTHERN RESEARCH INSTITUTE		N01CM57000 D.T.P.			335,000
PHARMACOLOGY/TOXICOLOGY			* 70.00*	234,500	
SPECIAL TOXICITY STUDIES					
TREATMENT STUDIES			* 30.00*	100,500	
CHEMOTHERAPY					

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
SOUTHERN RESEARCH INSTITUTE	N01CM43756 D.T.P.			2,300,000
ACQUISITION OF MATERIALS		* 1.00*	23,000	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 32.00*	736,000	
PRIMARY SCREENING, IN VITRO		4.00	92,000	
PRIMARY SCREENING, IN VIVO		16.00	368,000	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		10.00	230,000	
ANALOG SCREENING		2.00	46,000	
VERIFICATION SCREEN		* 26.00*	598,000	
DETAILED DRUG EVALUATION, IN VIVO		14.00	322,000	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		10.00	230,000	
ANTIVIRAL ACTIVITY		2.00	46,000	
PHARMACOLOGY/TOXICOLOGY		* 2.00*	46,000	
SPECIAL TOXICITY STUDIES		1.00	23,000	
BIOASSAY-DRUG METAB.		1.00	23,000	
BIOLOGICAL STUDIES		* 1.00*	23,000	
CELL KINETICS				
TREATMENT STUDIES		* 38.00*	874,000	
COMB. MODAL. THERAPY		38.00	874,000	
COMB. MODAL. THERAPY - SURG.		17.00	391,000	
COMB. MODAL. THERAPY - CHEM.		21.00	483,000	
SOUTHERN RESEARCH INSTITUTE	N01CM43780 D.T.P.			209,592
PHARMACOLOGY/TOXICOLOGY		*100.00*	209,592	
PHARMACOLOGY				
SOUTHERN RESEARCH INSTITUTE	N01CM43762 D.T.P.			5,500
ACQUISITION OF MATERIALS		* 80.00*	4,400	
NEW AGENT PROCUREMENT, SYNTHETICS				
PROCUREMENT OF PRECLINICAL MATERIAL		* 20.00*	1,100	
SYNTHETICS				
SOUTHWEST FOUNDATION FOR RESEARCH &	N01CM67075 D.T.P.			106,153
ACQUISITION OF MATERIALS		* 5.00*	5,308	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 95.00*	100,845	
PRIMARY SCREENING, IN VITRO		90.00	95,538	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	5,308	
SPARTAN RESEARCH ANIMALS, INC.	N01CM50582 A.P.			113,880
ACQUISITION OF MATERIALS		* 20.00*	22,776	
ANIMALS				
BASIC SCREEN		* 55.00*	62,634	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	28,470	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ANIMALS				
STANFORD RESEARCH INSTITUTE	N01CM33723			90,868
	D.T.P.			
PROCUREMENT OF PRECLINICAL MATERIAL		* 20.00*	18,174	
ANALYTICAL AND QUALITY CONTROL				
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	72,694	
ANALYTICAL AND QUALITY CONTROL				
STANFORD RESEARCH INSTITUTE	N01CM63776			160,000
	D.T.P.			
PROCUREMENT OF PRECLINICAL MATERIAL		* 80.00*	128,000	
RADIOLABELED MATERIALS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 20.00*	32,000	
RADIOLABEL				
STANFORD RESEARCH INSTITUTE	N01CM87207			194,000
	D.T.P.			
ACQUISITION OF MATERIALS		*100.00*	194,000	
NEW AGENT PROCUREMENT, SYNTHETICS				
STARKS ASSOCIATES, INC.	N01CM53745			212,288
	D.T.P.			
ACQUISITION OF MATERIALS		*100.00*	212,288	
NEW AGENT PROCUREMENT, SYNTHETICS		70.00	148,602	
DATA PROCESSING AND SUPPORT		30.00	63,686	
STARKS ASSOCIATES, INC.	N01CM53769			195,823
	D.T.P.			
PROCUREMENT OF PRECLINICAL MATERIAL		* 75.00*	146,867	
SYNTHETICS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 25.00*	48,956	
PRODUCTION, SYNTHETICS				
STARKS ASSOCIATES, INC.	N01CM87203			435,248
	D.T.P.			
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	217,624	
SYNTHETICS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	217,624	
PRODUCTION, SYNTHETICS				
STARKS ASSOCIATES, INC.	N01CM87206			187,421
	D.T.P.			
ACQUISITION OF MATERIALS		*100.00*	187,421	
NEW AGENT PROCUREMENT, SYNTHETICS		70.00	131,195	
DATA PROCESSING AND SUPPORT		30.00	56,226	
STEHLIN FOUNDATION FOR CANCER RESEARCH	N01CM67073			344,390
	D.T.P.			
BASIC SCREEN		*100.00*	344,390	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL				

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
TACONIC FARMS		N01CM50597 A.P.			184,600
ACQUISITION OF MATERIALS			* 20.00*	36,920	
ANIMALS					
BASIC SCREEN			* 55.00*	101,530	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	46,150	
ANIMALS					
TENNESSEE, UNIVERSITY OF		N01CM57021 CTEP			107,968
PROGRAM MANAGEMENT			* 10.00*	10,797	
ADMINISTRATION					
PHASE II CLINICAL TRIALS			* 30.00*	32,390	
CNS TUMORS	CHEM.				
PHASE III CLINICAL TRIALS			* 60.00*	64,781	
CNS TUMORS	RAD.		30.00	32,390	
CNS TUMORS	CHEM.		30.00	32,390	
TEXAS, UNIVERSITY OF, M.D. ANDERSON		N01CM33710 CTEP			192,831
PHASE III CLINICAL TRIALS			*100.00*	192,831	
OVARIAN	CHEM.				
TEXAS, UNIVERSITY OF, M.D. ANDERSON		N01CM77149 CTEP			144,916
PHASE II CLINICAL TRIALS			* 20.00*	28,983	
LUNG	RAD.		10.00	14,492	
LUNG	CHEM.		10.00	14,492	
PHASE III CLINICAL TRIALS			* 60.00*	86,950	
LUNG	RAD.		30.00	43,475	
LUNG	CHEM.		30.00	43,475	
PHASE IV CLINICAL TRIALS			* 20.00*	28,983	
LUNG	RAD.		10.00	14,492	
LUNG	CHEM.		10.00	14,492	
TEXAS, UNIVERSITY OF, MEDICAL BRANCH		N01CM87221 CTEP			95,072
PHASE IV CLINICAL TRIALS			*100.00*	95,072	
HEAD AND NECK	SURG.		30.00	28,522	
HEAD AND NECK	RAD.		30.00	28,522	
HEAD AND NECK	CHEM.		20.00	19,014	
HEAD AND NECK	SUPPORTIVE CARE		20.00	19,014	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM67116 CTEP			92,738
PHASE III CLINICAL TRIALS			* 50.00*	46,369	
OVARIAN	SURG.		15.00	13,911	
OVARIAN	RAD.		15.00	13,911	
OVARIAN	CHEM.		20.00	18,548	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PHASE IV CLINICAL TRIALS			* 50.00*	46,369	
OVARIAN	SURG.		15.00	13,911	
OVARIAN	RAD.		15.00	13,911	
OVARIAN	CHEM.		20.00	18,548	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM57042 CTEP			658,23
PHASE II CLINICAL TRIALS			* 55.00*	362,027	
BREAST	CHEM.		15.00	98,735	
COLORECTAL	CHEM.		15.00	98,735	
HEAD AND NECK	CHEM.		5.00	32,912	
LUNG	CHEM.		5.00	32,912	
MELANOMA	CHEM.		10.00	65,823	
SARCOMAS (GEN.)	CHEM.		5.00	32,912	
PHASE III CLINICAL TRIALS			* 45.00*	296,204	
BREAST	CHEM.		10.00	65,823	
COLORECTAL	CHEM.		5.00	32,912	
HEAD AND NECK	CHEM.		5.00	32,912	
LUNG	CHEM.		5.00	32,912	
MELANOMA	CHEM.		15.00	98,735	
SARCOMAS (GEN.)	CHEM.		5.00	32,912	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM67026 CTEP			55,23
PHASE III CLINICAL TRIALS			* 50.00*	27,617	
OVARIAN	SURG.		15.00	8,285	
OVARIAN	RAD.		15.00	8,285	
OVARIAN	CHEM.		20.00	11,047	
PHASE IV CLINICAL TRIALS			* 50.00*	27,617	
OVARIAN	SURG.		15.00	8,285	
OVARIAN	RAD.		15.00	8,285	
OVARIAN	CHEM.		20.00	11,047	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM43801 CTEP			43,75
PHASE I CLINICAL TRIALS			*100.00*	43,750	
BREAST	CHEM.		10.00	4,375	
COLORECTAL	CHEM.		10.00	4,375	
HEAD AND NECK	CHEM.		10.00	4,375	
KIDNEY	CHEM.		10.00	4,375	
LUNG	CHEM.		10.00	4,375	
MELANOMA	CHEM.		15.00	6,563	
PANCREATIC	CHEM.		10.00	4,375	
SARCOMAS (GEN.)	CHEM.		10.00	4,375	
STOMACH	CHEM.		10.00	4,375	
NON-SPECIFIC	CHEM.		5.00	2,188	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM77153 CTEP			130,83
PHASE II CLINICAL TRIALS			* 20.00*	26,166	
LUNG	RAD.		10.00	13,083	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
LUNG	CHEM.		10.00	13,083	
PHASE III CLINICAL TRIALS			* 60.00*	78,499	
LUNG	RAD.		30.00	39,249	
LUNG	CHEM.		30.00	39,249	
PHASE IV CLINICAL TRIALS			* 20.00*	26,166	
LUNG	RAD.		10.00	13,083	
LUNG	CHEM.		10.00	13,083	
TEXAS, UNIVERSITY OF		N01CM53767 D.T.P.			84,357
VERIFICATION SCREEN			*100.00*	84,357	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL					
TEXAS, UNIVERSITY OF		N01CM53832 C.O.P.			650,000
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	650,000	
LEUK.-ACUTE GRAN.	PROTECTED ENVIRON.		20.00	130,000	
LEUK.-ACUTE LYMPH.	PROTECTED ENVIRON.		40.00	260,000	
SARCOMAS (GEN.)	PROTECTED ENVIRON.		40.00	260,000	
UPJOHN COMPANY		N01CM77100 D.T.P.			963,370
ACQUISITION OF MATERIALS			* 85.00*	818,865	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			83.00	799,597	
DATA PROCESSING AND SUPPORT			2.00	19,267	
BASIC SCREEN			* 15.00*	144,506	
PRIMARY SCREENING, IN VITRO			10.00	96,337	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			5.00	48,169	
UTAH, UNIVERSITY OF		N01CM77142 D.T.P.			195,000
ACQUISITION OF MATERIALS			*100.00*	195,000	
NEW AGENT PROCUREMENT, SYNTHETICS					
VALUE ENGINEERING COMPANY		N01CM67086 D.T.P.			908,495
ACQUISITION OF MATERIALS			* 14.00*	127,189	
BIOASSAY OF NATURAL PRODUCTS					
BASIC SCREEN			* 58.00*	526,927	
PRIMARY SCREENING, IN VITRO			5.00	45,425	
PRIMARY SCREENING, IN VIVO			53.00	481,502	
VERIFICATION SCREEN			* 20.00*	181,699	
DETAILED DRUG EVALUATION, IN VIVO					
TREATMENT STUDIES			* 8.00*	72,680	
CHEMOTHERAPY					
VANDERBILT UNIVERSITY MEDICAL		N01CM77122 CTEP			114,072
PHASE II CLINICAL TRIALS			* 20.00*	22,814	
LUNG	RAD.		10.00	11,407	
LUNG	CHEM.		10.00	11,407	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PHASE III CLINICAL TRIALS			* 60.00*	68,443	
LUNG	RAD.		30.00	34,222	
LUNG	CHEM.		30.00	34,222	
PHASE IV CLINICAL TRIALS			* 20.00*	22,814	
LUNG	RAD.		10.00	11,407	
LUNG	CHEM.		10.00	11,407	
VERMONT, UNIVERSITY OF, COLLEGE OF		N01CM33716 BCRC			42,000
PROGRAM MANAGEMENT			* 20.00*	8,400	
ADMINISTRATION			1.00	420	
COMMUNICATION AND EDUCATION			9.00	3,780	
DATA PROCESSING AND SUPPORT			10.00	4,200	
PHASE III CLINICAL TRIALS			* 80.00*	33,600	
LEUK.-ACUTE GRAN.	SUPPORTIVE CARE		50.00	21,000	
NON-SPECIFIC	CHEM.		30.00	12,600	
VETERANS ADMINISTRATION		Y01CM70106 C.O.P.			2,349,817
PHASE III CLINICAL TRIALS			* 40.00*	939,927	
HEAD AND NECK	CHEM.		5.00	117,491	
HEAD AND NECK	IMM.		5.00	117,491	
LUNG	RAD.		10.00	234,982	
LUNG	CHEM.		20.00	469,963	
PHASE IV CLINICAL TRIALS			* 40.00*	939,927	
COLORECTAL	SURG.		5.00	117,491	
COLORECTAL	RAD.		5.00	117,491	
COLORECTAL	CHEM.		5.00	117,491	
ESOPHAGEAL	RAD.		2.50	58,745	
ESOPHAGEAL	CHEM.		2.50	58,745	
LUNG	SURG.		5.00	117,491	
LUNG	CHEM.		5.00	117,491	
LUNG	IMM.		5.00	117,491	
PANCREATIC	CHEM.		2.50	58,745	
STOMACH	CHEM.		2.50	58,745	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	469,963	
COLORECTAL	DATA PROC. & SUPP.		2.50	58,745	
ESOPHAGEAL	DATA PROC. & SUPP.		2.50	58,745	
HEAD AND NECK	DATA PROC. & SUPP.		2.50	58,745	
LUNG	DATA PROC. & SUPP.		5.00	117,491	
PANCREATIC	DATA PROC. & SUPP.		2.50	58,745	
STOMACH	DATA PROC. & SUPP.		5.00	117,491	
VETERANS ADMINISTRATION		Y01CM70107 CTEP			1,282,047
PHASE III CLINICAL TRIALS			* 40.00*	512,819	
HEAD AND NECK	CHEM.		5.00	64,102	
HEAD AND NECK	IMM.		5.00	64,102	
LUNG	RAD.		10.00	128,205	
LUNG	CHEM.		20.00	256,409	
PHASE IV CLINICAL TRIALS			* 40.00*	512,819	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
COLORECTAL	SURG.		5.00	64,102	
COLORECTAL	RAD.		5.00	64,102	
COLORECTAL	CHEM.		5.00	64,102	
ESOPHAGEAL	RAD.		2.50	32,051	
ESOPHAGEAL	CHEM.		2.50	32,051	
LUNG	SURG.		5.00	64,102	
LUNG	CHEM.		5.00	64,102	
LUNG	IMM.		5.00	64,102	
PANCREATIC	CHEM.		2.50	32,051	
STOMACH	CHEM.		2.50	32,051	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	256,409	
COLORECTAL	DATA PROC. & SUPP.		2.50	32,051	
ESOPHAGEAL	DATA PROC. & SUPP.		2.50	32,051	
HEAD AND NECK	DATA PROC. & SUPP.		2.50	32,051	
LUNG	DATA PROC. & SUPP.		5.00	64,102	
PANCREATIC	DATA PROC. & SUPP.		2.50	32,051	
STOMACH	DATA PROC. & SUPP.		5.00	64,102	
VETERANS ADMINISTRATION		Y01CM60102 D.T.P.			37,020
ACQUISITION OF MATERIALS			* 90.00*	33,318	
NEW AGENT PROCUREMENT, SYNTHETICS					
PROCUREMENT OF PRECLINICAL MATERIAL			* 10.00*	3,702	
SYNTHETICS					
WALTER REED ARMY MEDICAL CENTER		Y01CM80108 CTEP			67,425
BIOLOGICAL STUDIES			*100.00*	67,425	
DIAGNOSIS					
WARF INSTITUTE, INC.		N01CM53770 D.T.P.			422,901
ACQUISITION OF MATERIALS			* 50.00*	211,451	
BIOASSAY OF NATURAL PRODUCTS					
BASIC SCREEN			* 40.00*	169,160	
PRIMARY SCREENING, IN VIVO			36.00	152,244	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			4.00	16,916	
VERIFICATION SCREEN			* 9.00*	38,061	
DETAILED DRUG EVALUATION, IN VIVO					
TREATMENT STUDIES			* 1.00*	4,229	
CHEMOTHERAPY					
WASHINGTON UNIVERSITY		N01HB62970 C.D.			53,465
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	53,465	
OTHER	TRANSFUSION RES.				
WAYNE STATE UNIVERSITY		N01CM67105 CTEP			348,456
PHASE II CLINICAL TRIALS			* 55.00*	191,651	
BREAST	CHEM.		15.00	52,268	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
COLORECTAL	CHEM.		15.00	52,268	
HEAD AND NECK	CHEM.		5.00	17,423	
LUNG	CHEM.		5.00	17,423	
MELANOMA	CHEM.		10.00	34,846	
SARCOMAS (GEN.)	CHEM.		5.00	17,423	
PHASE III CLINICAL TRIALS			* 45.00*	156,805	
BREAST	CHEM.		10.00	34,846	
COLORECTAL	CHEM.		5.00	17,423	
HEAD AND NECK	CHEM.		5.00	17,423	
LUNG	CHEM.		5.00	17,423	
MELANOMA	CHEM.		15.00	52,268	
SARCOMAS (GEN.)	CHEM.		5.00	17,423	
WISCONSIN, UNIVERSITY OF		N01CM67076 D.T.P.			59,624
ACQUISITION OF MATERIALS			* 5.00*	2,981	
BIOASSAY OF NATURAL PRODUCTS					
BASIC SCREEN			* 95.00*	56,643	
PRIMARY SCREENING, IN VITRO			90.00	53,662	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			5.00	2,981	
YALE UNIVERSITY SCHOOL OF MEDICINE		N01CM43797 CTEP			144,900
PHASE II CLINICAL TRIALS			* 30.00*	43,470	
PANCREATIC	CHEM.				
PHASE IV CLINICAL TRIALS			* 50.00*	72,450	
PANCREATIC	SURG.		5.00	7,245	
PANCREATIC	RAD.		20.00	28,980	
PANCREATIC	CHEM.		25.00	36,225	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	28,980	
PANCREATIC	DATA PROC. & SUPP.				
YALE UNIVERSITY SCHOOL OF MEDICINE		N01CM53843 CTEP			105,000
PHASE IV CLINICAL TRIALS			* 90.00*	94,500	
STOMACH	SURG.		25.00	26,250	
STOMACH	RAD.		25.00	26,250	
STOMACH	CHEM.		40.00	42,000	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	10,500	
STOMACH	MARKERS				

TABLE IV
DESCRIPTION OF CONTRACTS
IN THE
DIVISION OF CANCER TREATMENT PROGRAM

ABBOTT LABORATORIES (N01-CM4-3704)

This contract was designed to develop a conditioning medium that will cause normal and leukemic granulocytic precursor cells to differentiate and form colonies of mature granulocytes. This "colony stimulating factor" has now been isolated, purified and characterized. The terminal year of this contract will be spent preparing enough material for a clinical trial in 50 patients. An IND is being submitted.

AGRICULTURE, DEPARTMENT OF (TRANSFER OF FUNDS) (Y01-CM4-0001)

Under the Transfer of Funds Agreement with the Medicinal Plant Resources Laboratory of the U.S.D.A., worldwide general plant collections and recollections are made. The general plant collections (approximately 100 plants) are shipped to WARF Institute, Inc. for extraction and the extracts are screened *in vitro* and *in vivo* for antineoplastic activity. Confirmed active plants are recollected in large quantities (300 to 500 lbs.) on a priority based on the needs of the Division of Cancer Treatment Program for shipment to the fractionating chemists. Very large recollections are made, as requested by the Developmental Therapeutics Program, for the preparation of clinical quantities of drugs. The nomenclature of all plants acquisitioned is being continuously reviewed and corrected, and a computer file of all plants is being maintained.

ALABAMA, UNIVERSITY OF (N01-CM6-7083)

This contractor operates primarily at the genetic center level, making certain that isolator reared progeny from the caesarian-derived animals contain no undesirable organisms. Contractor also coordinates virus diagnostic results with his own observations of pathological lesions in arriving at a definitive diagnosis when disease problems occur. This contract will be funded by DCCP.

ALBANY MEDICAL COLLEGE (N01-CM5-7032)

This contract is designed to support prospective, randomized controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

ALBERT EINSTEIN COLLEGE OF MEDICINE (N01-CM4-3787)

The major emphasis of this project is to provide a detailed evaluation of new antitumor drugs which affect protein synthesis. For drugs having significant

ALBERT EINSTEIN COLLEGE OF MEDICINE (N01-CM4-3787) (CONTINUED)

effects on protein synthesis, detailed studies on messenger binding, initiation, translocation and peptide bond formation will be done. In addition, detailed studies on the mechanism of action of new antitumor drugs which may directly affect the structure of DNA are performed. This contract terminated March 1978.

ALDRICH CHEMICAL COMPANY, INC. (N01-CM8-7202)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 30% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

AMERICAN COLLEGE OF RADIOLOGY (RTOG) (N01-CM8-7175)

This contract is to establish a registry of radiation therapy complications. Specifically, this work involves the establishment of a comprehensive data bank relating to radiation therapy complications, long-term effects, and radiation pathology. The registry will consist of an epidemiological center and a radiation pathology reference center. The data bank will serve as a resource for cooperative clinical trial groups as well as the radiation therapy community at large to both retrospectively and prospectively register cases of radiation injury and second malignancies. The epidemiological center will also function to determine the effects of dose modification, influence of radiation modifiers, and the effects of concurrent medical conditions on the likelihood of development of complications. The radiation pathology reference center will document and analyze histologic material, radiation injuries secondary to treatment, maintain a registry of pathology materials for study and training, and correlate and quantitate time-dose data to develop accurate dose-response curves and radiation tolerance levels.

AMERICAN TYPE CULTURE COLLECTION (N01-CM8-7158)

This contract has three main objectives: (1) to provide substantial quantities (400 grams per annum) of tissue cultured normal and malignant mammalian cells; (2) to receive, process, analyze, distribute and store up to 200 fresh human blood samples, principally from leukemic patients; and (3) to grow normal and leukemic human leukocytes in short- and long-term culture under conditions specified by the GPO. The accomplishment of the above tasks requires the maintenance of strict quality control procedures, of accessible investories of certified cellular materials and of appropriate biohazard safeguards and the performance of selected analytical procedures, including cellular cytochemistry, cell pulse-labelling procedures, and DNA polymerase assays on culture media concentrates.

ARIZONA STATE UNIVERSITY (N01-CM6-7048)

The major objective of this contract is the preparation, from animal materials of marine species, of extracts suitable for bioassay in the National Cancer Institute antitumor screen. The extracts are prepared in accordance with procedures acceptable to the National Cancer Institute. Extracts showing preliminary activity are prepared anew for confirmatory testing and materials showing confirmed activity are assigned for fractionation and isolation. This contract is not being renewed.

ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE (Y01-CM6-0105)

This Interagency Agreement was undertaken to test and evaluate the safety and performance of whole body hyperthermia equipment purchased by the Division of Cancer Treatment from Webb Associates, Inc. The approach was to first determine the thermal properties of the Webb equipment with a copper mannequin, previously developed for military equipment testing. The mannequin is computer controlled to generate and conduct heat in a manner simulating man. The results of the mannequin tests were used in an existing mathematical model of human physiological response to heat stress under extreme conditions. The physiological model with the measured thermal parameters of the Webb equipment was then verified by the use of the Webb equipment on a series of 13 normal human volunteers. The physician who conducted the human volunteer trials of the Webb equipment was then assigned to NCI for a period of nine months to assist NCI clinical personnel in the use of the Webb equipment for whole body hyperthermia treatment of cancer patients. Preliminary recommendations and a final report were issued to NCI and results of the physiological model verification were published in the American Journal of Physiology. All work was completed January 1978.

ARS/SPRAGUE-DAWLEY (N01-CM5-3813)

This primary genetic center produces a variety of outbred, inbred and hybrids of inbred rodents. All production activities are effected in a closely controlled environment.

ARS/SPRAGUE-DAWLEY (N01-CM5-3846)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. Major emphasis is upon the production of C57BL/6, C3H/He MTV+, DBA/8, BALB/c-CMC, and CD8F1 mice.

ARTEK SYSTEMS CORPORATION (N01-CM7-7128)

This contract provides unique, semi-automated instruments for rapid processing of radioautographic slide preparations for measuring grain counts, integrated optical density, cytoplasmic and nuclear areas, and perimeters of cells. Such instruments will potentially support intramural and extramural activities in cell kinetic studies of the DCT Program.

ARTHUR D. LITTLE, INC. (N01-CM3-3727)

This project supplies biostatistical support to the Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, specifically to aid in establishing parameters and criteria of activity and toxicity in current and new antitumor screens, in formulating detailed experimental protocols for use in contract testing laboratories, and in maintaining testing quality control by evaluating screening methodology with respect to test-to-test and lab-to-lab variability. The workscope includes studies of individual screening models (animal tumor assays) with regard to their validity (e.g., reproducibility) and their yield of active materials and to determine the degree of correlations among alternative models. Additionally, this project includes tasks related to the evaluation and summarization of clinical results. Analysis of data from antitumor screening laboratories and from the clinic as well provides for the quantitative assessment of correlations between activity against specific laboratory models and activity against specific human malignancies.

ARTHUR D. LITTLE, INC. (N01-CM5-3765)

This contract employs a variety of animal tumor systems in carrying out its three major objectives: (1) evaluation of analogs of known antitumor drugs; (2) combination chemotherapy studies; and (3) combined modality studies involving drugs plus radiation and for surgery. To assess potential superiority of analogs, direct comparison tests of analogs and the parent compound are carried out in systems designed for each compound class of interest. The results of these structure-activity tests are reported rapidly to NCI and to the suppliers to guide future synthetic efforts. Combinations of drugs are examined in both leukemic and solid tumor models to determine their potential superiority over the drugs used alone. Finally, considerable emphasis is placed on radiation therapy and combinations of radiation sensitizers and radiation potentiators with radiation.

ARTHUR D. LITTLE, INC. (N01-CM5-7006)

This contract provides in vivo screening of new synthetics and natural products and carries out the secondary evaluation of those materials demonstrating activity in the primary screen. All testing is carried out in accordance with the Developmental Therapeutics Program protocols. New materials are tested initially against the P388 leukemia test system.

ARTHUR D. LITTLE, INC. (N01-CM5-7006) (CONTINUED)

Testing is also carried out in the panel of antitumor systems when requested by Drug Evaluation Branch staff. Detailed testing, such as schedule dependency studies in the active tumor system, is conducted with materials of potential clinical interest. Methodology studies, primarily the development, characterization and evaluation of tumor test systems, are carried out as directed by the Project Officer. The current level of testing is approximately 25,000 L1210 equivalent tests per year.

ARTHUR D. LITTLE, INC. (N01-CM6-3823)

This contract provides broad biomedical engineering resources which respond to individual research projects within the Division of Cancer Treatment. A flexible mechanism of support defines and implements exploratory engineering projects in close collaboration with the DCT staff in diverse areas covering instrumentation and mathematical modeling for tumor cell kinetics, automated microspectrometry, novel clinical equipment development, and thermometry for hyperthermia studies, etc. A "core group" of senior engineers continually consults and interacts with the DCT staff on the definition of problems and projects while "project groups" are tailored to implement specific engineering tasks under NCI staff direction. This contract will terminate October 1978.

ARTHUR D. LITTLE, INC. (N01-CM6-7047)

The workscope of this program is to extend the research objectives of the Central Nervous System Solid Tumor Studies with the neonate-puppy animal model. This will include the transplantation of gliomas in the CNS of neonate beagle puppies to provide suitable tumor materials for chemotherapy studies; chemotherapy studies with selected agents against the transplantable gliosarcoma in the brains of newborn puppies; histopathologic evaluation of the effects of chemotherapeutic agents against the transplantable gliosarcoma in the brains of newborn puppies; collaborative studies with clinical and pharmacology groups of the National Cancer Institute to study the growth kinetics and biologic behavior of transplantable gliomas to selected agents; methodological studies as agreed upon with the approval of the Project Officer and exploring the use of the transplantable beagle tumor in multimodality therapy. This contract will terminate December 1978.

ARTHUR D. LITTLE, INC. (N01-CM6-7078)

Due to the need of testing capacity for the *in vitro* testing of crude natural products, continuation of analogue evaluation (see Contract N01-CM5-3765), and new synthetic materials submitted in too small a quantity for *in vivo* evaluation, this service contract was awarded. *In vitro* testing in the 9KB and P388 tumor systems was conducted until mid November at which time it was determined by Drug Evaluation Branch staff that this contract, scheduled for termination February 1978, would best serve the Developmental Therapeutics Program by discontinuing 9KB and P388 testing. In their place, studies

ARTHUR D. LITTLE, INC. (N01-CM6-7078) (CONTINUED)

toward the establishment of other *in vitro* systems, that could be used in the isolation of the active moiety of crude natural products, were undertaken. This effort will continue until the end of 1978 with no additional funding as a result of the change in work scope.

ARTHUR D. LITTLE, INC. (N01-CM8-7163)

This contract encompasses two distinct work areas: (1) physiological disposition and biochemical pharmacology, and (2) electron microscopy and radioautography. Under the first activity, work is conducted on the physiological disposition and biochemical pharmacology of antitumor agents under active development in the Division of Cancer Treatment Program. Work has been conducted on PALA, ftorafur, dichlorolapachol, anthracyclines, ellipticine, 5-azacytidine plus THU and selenoguanosine. Electron microscopy and radioautography have focused on the whole body radioautography of AMSA, ICRF-159, hexamethylmelamine, inosine dialdehyde, procarbazine and d-tetrandrine. Electron microscopy has been conducted on the effect of Vitamin E on the adriamycin-induced cardiomyopathy in mouse heart.

ASH STEVENS, INC. (N01-CM5-7012)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts required. About 75% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

ASH STEVENS, INC. (N01-CM8-7204)

This service preparative contract is for the resynthesis of bulk chemicals and drugs required for completion of drug evaluation studies, with approximately 30% of the effort being devoted to the production of clinical materials. The materials assigned for resynthesis are not readily available in the quantities needed from the original supplier or on the open market. Preparations vary in quantity from gram to multikilogram scale.

ASSOCIATED BIOMEDIC SYSTEMS, INC. (N01-CM0-2271)

The purposes of this contract are as follows: (1) to supply large quantities of normal human blood lymphocytes, untreated and stimulated with phytohemagglutinin (PHA); (2) to prepare large quantities of PHA-stimulated lymphocyte conditioned medium to be utilized for purification of myeloid colony stimulating activity (CSA) and of T-lymphocyte stimulating activity; (3) to produce large quantities of cultured human leukocyte (lymphoid and

ASSOCIATED BIOMEDIC SYSTEMS, INC. (N01-CM0-2271) (CONTINUED)

myeloid) suspension cultured cells; (4) to initiate selected new lymphoblastoid cell lines; and (5) to perform tests to characterize fresh and cultured human leukocytes, including HLA type, antigen tests for EBV, cell surface immunoglobulins and erythrocyte rosette tests. This contract will be phased out in September 1978.

BATTELLE MEMORIAL INSTITUTE (N01-CM4-3746)

In October 1977, after recompetition, a Prime contract was renegotiated with Battelle Memorial Laboratories for supervision of contracts carrying out the toxicologic evaluation of potential oncolytic agents under the aegis of the Laboratory of Toxicology. Through the Prime contract mechanism, preclinical toxicologic studies of agents under consideration for potential clinical use are handled under a single management-type contract. The work scope is comprised of four tasks as follows: Task I - Full Protocol Studies; Task II - High Priority Toxicity Studies (i.e., any portion of the Protocol of the Laboratory of Toxicology); Task III - Specific Organ Testing; and Task IV - Automation of toxicity data, anomaly detection, scheduling of studies, and financial management.

BATTELLE MEMORIAL INSTITUTE (N01-CM5-7005)

Battelle Columbus Laboratories conduct *in vivo* testing of new synthetic materials and natural products of plant, animal and fermentation origin. In addition, tumor panel testing is conducted upon active materials of interest to the Developmental Therapeutics Program. This testing is conducted at a level of effort of approximately 50,000 L1210 equivalents per year. New synthetic and natural product materials are tested in the P388 pre-screen. Schedule dependency studies are conducted on materials in development toward clinical trial upon request.

BATTELLE MEMORIAL INSTITUTE (N01-CM6-7099)

This contract provides for the development of protocols and screening of compounds on human tumor xenografts in athymic mice. Currently, three working protocols are under study for a colon, mammary and lung xenograft system. Screening is proceeding and criteria are being further evaluated.

BELLAIRE ACRES (N01-CM5-0583)

This contract furnishes approximately 312,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

BEN VENUE LABORATORIES, INC. (N01-CM6-7109)

This service contract provides for the development and production of parenteral clinical dosage forms of antitumor agents. Ben Venue Laboratories, Inc. has the capacity for preparing large-scale production batches of dry-filled, liquid-filled and lyophilized sterile products. Specifically, the contractor performs the following services: (1) formulation development of sterile parenteral products; (2) production of sterile products; (3) quality assurance testing of finished products; and (4) stability surveillance of all dosage forms produced. All products are packaged, labelled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

BIOTECH RESEARCH LABORATORIES, INC. (N01-CM6-7052)

This contract has the objective of implementing and operating an in vitro primary screen for identification of drugs with potential therapeutic activity in established tumors and in the remission maintenance of treated tumors. Specifically the screen is designed to detect (a) drug activity against cells with oncogenic virus gene-directed phenotypic properties, and (b) drug activity against those oncogenic virus properties associated with tumor induction of reinduction.

BOWMAN GRAY SCHOOL OF MEDICINE (N01-CM6-7054)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

BRIGHAM YOUNG UNIVERSITY (N01-CM4-3790)

The major emphasis of this project is detailed evaluation of selected new antitumor drugs on folic acid metabolism. In addition to a study of drug action on thymidylate synthetase and dihydrofolate reductase, drug effects on serine transhydroxymethylase, N^5, N^{10}-methylene THF reductase, N^5-methyl

BRIGHAM YOUNG UNIVERSITY (N01-CM4-3790) (CONTINUED)

THF homocyteine transmethylase, N^5, N^{10}-methylene THF dehydrogenase, N^5, N^{10}-methenyl THF cyclohydrase, 5-amino, 4-imidazolecarboxamide ribonucleotide transformylase and glycinamide ribonucleotide transformylase will be studied. This contract terminated March 1978.

BRISTOL-MYERS COMPANY (N01-CM3-3751)

This is a no-cost contract concerned with the marketing of the clinically useful nitrosourea, BCNU. The Contractor summarized the clinical and other data, and prepared and filed the New Drug Application (NDA) with the Food and Drug Administration. The NDA has been approved, and the drug is currently on the market.

BRISTOL-MYERS COMPANY (N01-CM6-7051)

This is a no-cost contract concerned with the marketing of the clinically useful nitrosourea, CCNU. The Contractor summarized the clinical and other data, and prepared and filed the New Drug Application (NDA) with the Food and Drug Administration. The NDA has been approved, and the drug is currently on the market.

BRISTOL-MYERS COMPANY (N01-CM7-7138)

The major objective of this fermentation contract is the preparation of novel antibiotics. These new antibiotics are obtained using the fermentation techniques of biotransformation and co-metabolism of antibiotics, chemicals and plant materials. This effort is to convert materials which have solubility, toxicity or marginal activity problems into worthwhile antineoplastic agents. A multitude of unique organisms are isolated and used to obtain these microbial conversions in an effort to obtain new antineoplastic agents. This year the biotransformation of eight compounds was studied in detail with each compound being exposed to 190 or more different microorganisms. Twenty metabolites were produced and are being isolated to determine their biological activity.

BRISTOL-MYERS COMPANY (N01-CM7-7147)

This fermentation contract is designed primarily to obtain novel antitumor agents. This contract includes: (1) the preparation of fermentation beers from various unique microbes isolated in Bristol's Japanese facility; (2) eight different *in vitro* pre-screens are used to evaluate the fermentations; (3) development of an *in vitro* assay to assist in quickly isolating the active anticancer agents; (4) dereplication of the materials to determine novelty; (5) chemical isolation of the active component; and (6) production of large quantities of new agents to thoroughly evaluate them in DCT screens.

BRITISH COLUMBIA, UNIVERSITY OF (N01-CM2-3223)

The contractor's research is aimed to find practical methods for the synthesis of vincristine and vinblastine which would allow easy and variable access to structurally related analogs. The work involves studies with a variety of chemical approaches to accomplish the required subtle interconversions of indole and bisindole alkaloids. Biogenetic synthesis also is investigated as a viable source of complicated alkaloid structures needed as starting materials for the total synthesis.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM0-2092)

This contract was designed to provide HL-A typing analyses on patients and their families as well as typing of selected donors from the Clinical Center Blood Bank and all platelet donors from the Central Blood Service of Baltimore. Typing data on over 15,000 NIH donors and recipients are on computer file. All typings are performed and entered into the computerized file on the same day that the blood was received. Thus, HL-A typing results for persons bled in Bethesda are consistently available through the computer terminal by the end of the next day. An average of 600 typings are performed annually.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM4-3791)

The goal of this project is to develop and use assays which are capable of detecting and evaluating antitumor agents which can increase the lethal effects of ionizing radiation in vitro and in vivo. Such effects will be evaluated by cytocidal activity in cell culture, by effects on the cell's ability to repair x-ray induced DNA damage and by effects on the growth of locally implanted Lewis lung carcinoma in vivo. This contract terminated April 1978.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM4-3798)

This contract was designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas. It is presently being phased out.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM5-3752)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM5-3752) (CONTINUED)

Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated. This contract will expire June 1978.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM6-7097)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM7-7151)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage 1 tumors are randomized (double blind trial) to intrapleural BCG + INH x 12 weeks versus placebo intrapleural BCG + placebo INH x 12 weeks. Resectable Stage II and III epidermoid carcinoma is randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-HB6-2971)

This contract is concerned with clinical studies to determine the efficacy of granulocyte transfusions and in what patient population they are useful.

CALIFORNIA, UNIVERSITY OF (SAN FRANCISCO) (N01-CM6-7055)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group.

CATHOLIC MEDICAL CENTER OF BROOKLYN & QUEENS, INC. (N01-CM6-7081)

This contract provides an experimental mouse model for human breast cancer therapy. The purpose of the contract is to (1) screen for new agents, (2) study drug combinations, and (3) study drugs combined with surgery and/or immunotherapy. CD8F1 hybrid mice bearing spontaneous breast tumors are used in the combined modality studies and in evaluating high priority compounds. Drug screening studies are performed in CD8F1 mice bearing 24-hour or advanced first generation tumor transplants. Agents to be screened are selected according to the following criteria: activity in the P388 pre-screen, biochemical rationales, activity reported in the literature, candidates for clinical trial, and agents with established clinical activity.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-004-1)

The main objective of the Agreement is to procure a variety of compounds in sufficient quantities for anticancer evaluation. These materials arise from several sources: (a) synthesis of aromatic compounds containing a variety of functionalities such as nitro, isothiocyano, etc.; (b) synthesis of nitrogen and nitrogen-sulfur heterocyclics; (c) the modification of natural products with anticancer activity, for example, alkaloids, antibiotics, peptides, etc.; and (d) collection extraction, and isolation of Indian Medicinal plants. In addition, selected compounds from their internal programs in other disease areas are made available to us for anticancer evaluation.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-004-N)

The Central Drug Research Institute, Lucknow, India, has several components of the project related to natural products as follows: 1) collects native Indian plants and prepares extracts for antitumor screening; 2) carries out isolation and structural studies of antitumor agents from active plants; 3) synthesizes analogs of natural products such as emetine, camptothecin actinomycin and peptide antibiotics; and 4) engages in studies of microencapsulation and other areas related to drug formulation and release.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM5-0598)

This procurement contract is designed to furnish 312,000 six-week-old CD2F1 (BALB/c female x DBA/2 male) hybrid mice free of pseudomonads for the Developmental Therapeutics Program contract studies. The breeding animals originate from the genetic center at this site.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM5-3848)

This rodent production center contract supports a production effort designed to furnish animals as required by laboratory programs. Breeding animals are furnished by the Government.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM7-7141)

This primary genetic center has as its objectives the development of associated foundation colonies of inbred rodents required for program studies. Pedigreed animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of organisms and are developed as pedigreed expansion colonies in a variety of isolators. Offspring from this second stage are issued to secondary genetic centers which, in turn, produce breeding animals for large-scale production colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring. A large-scale production colony is maintained in order to provide suitable numbers of rodents for laboratory investigators. Task I is concerned with the production of germ-free rodents, counterparts with defined microbial flora and large-scale production; Task 2 in Calco, Italy, produces rodents in bio-containment environments; and Tasks 3 and 4 are concerned with the production of rodents for specific investigations.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM8-7199)

This contract provides for the maintenance of a Strain 2 guinea pig associated isolator foundation colony. In addition, a small expansion colony will be maintained within a barrier environment. This contract is funded by the Office of the Director, NCI.

CHEMICAL ABSTRACTS SERVICE (N01-CM4-3722)

The Chemical Abstracts Service (CAS) has participated in installing and is operating the new Chemical Information System which supports the Developmental Therapeutics Program's accessioning and screening activities. This system provides the improved capability to: (1) identify which accessions are new to the Program and which duplicate or are closely related to known active compounds; (2) produce computer-generated shipping documents and other communications to suppliers, screeners, in-house staff, and the acquisition and storage and distribution contractors who are involved in the accessioning and screening process; (3) maintain inventory control; (4) maintain management and operational control of the accessioning process; and (5) search the files on-line to secure an immediate answer for a possible duplicate sample, for analogs of a compound of interest, or for a management inquiry. CAS is evaluating and installing various system enhancements which will increase the effectiveness and efficiency of searching, reporting, and correcting the file. Support is provided in the development of a model to preselect for acquisition compounds which would have a high probability of activity in the prescreen based on statistical evaluation of the contribution to activity made by the chemical structural fragments of compounds in the Program's collection and the biological activity of these compounds. Limited support is also provided in identifying for acquisition compounds in the literature which display biological characteristics which may correlate with antitumor activity.

CHICAGO, UNIVERSITY OF (N01-CM6-7095)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

CHILDREN'S HOSPITAL MEDICAL CENTER (N01-CP6-5826)

As part of a multi-institutional cooperative effort, the effectiveness of routine nutritional practice will be compared with that of total parenteral nutrition via central-line hyperalimentation. Twenty-four pediatric patients with malignant tumors of the abdomen and/or pelvis requiring radiotherapy and intensive courses of multiple chemotherapeutic agents will be studied. Comparisons will be made with respect to: (a) tolerance to therapeutic schedule; (b) therapeutic response to combined chemotherapy and radiotherapy; (c) effectiveness of having the bowel "at rest" with respect to effects of therapy on gastrointestinal function; (d) nutritional status of the patient; and (e) complications during therapy. It is hypothesized that maintaining good nutritional status through total parenteral nutrition during combined radiotherapy to the abdomen and intensive chemotherapy, will result in fewer complications, decreased hospitalization time, and better therapeutic response. This hypothesis will be tested by a prospective randomized study comparing the patients maintained by total parenteral nutrition with those on routine dietary intake.

CHILDREN'S HOSPITAL OF LOS ANGELES (N01-CM4-3803)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies have been conducted on β-deoxythioguanosine (NSC-71851), neocarzinostatin (NSC-157365) and imidazole pyrazole (IMPY, NSC-51143) during the past year. This contract will be terminated September 1978.

CHILDREN'S HOSPITAL OF LOS ANGELES (N01-CP6-5828)

As part of a multi-institutional cooperative effort, the effectiveness of routine nutritional practice will be compared with that of total parenteral nutrition via central-line hyperalimentation. Thirty pediatric patients with malignant tumors of the abdomen and/or pelvis requiring radiotherapy and intensive courses of multiple chemotherapeutic agents will be studied. Comparisons will be made with respect to: (a) tolerance to therapeutic schedule; (b) therapeutic response to combined chemotherapy and radiotherapy; (c) effectiveness of having the bowel "at rest" with respect to effects of therapy on gastrointestinal function; (d) nutritional status of the patient; and (e) complications during therapy. It is hypothesized that maintaining good nutritional status through total parenteral nutrition during combined radiotherapy to the abdomen and intensive chemotherapy, will result in fewer

CHILDREN'S HOSPITAL OF LOS ANGELES (N01-CP6-5828) (CONTINUED)

complications, decreased hospitalization time, and better therapeutic response. This hypothesis will be tested by a prospective randomized study comparing the patients maintained by total parenteral nutrition with those on routine dietary intake.

CINCINNATI, UNIVERSITY OF (N01-CM8-7222)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The initial protocol is being written and will study the toxic effects as well as the therapeutic benefits of intensive preoperative chemotherapy and maintenance chemotherapy when added to a standard regimen of surgery and postoperative radiotherapy. Controls will receive the standard surgery/radiotherapy regimen alone. Study sites will be limited to the anterior tongue, floor of the mouth and retromolar trigone/anterior tonsillar pillar areas in the oral cavity, and glottic larynx, supraglottic larynx and pyriform sinus in the laryngopharyngeal region. Only patients with Stage III and IV lesions will be studied, except for patients with Stage II pyriform sinus lesions.

CLINICA NEUROCHIRURGICA DELL UNIVERSITA (DI PAVIA, ITALY) (N01-CM6-7056)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

COLLABORATIVE RESEARCH, INC. (N01-CM7-7071)

This contract has as one of its objectives clarification of the metabolic basis of selectivity and therapeutic effectiveness of cyclophosphamide. Another objective is the development of new analogs of cyclophosphamide and its metabolites and precursors with a broader spectrum of activity and

COLLABORATIVE RESEARCH, INC. (N01-CM7-7071) (CONTINUED)

enhanced antitumor activity. The specific compounds selected for synthesis are expected to define: a) the role of activation on the biological activity; b) the transport roles of activated intermediates; and c) the role of selective detoxification on antitumor activity.

COLORADO, UNIVERSITY OF, MEDICAL CENTER (N01-CM1-2186)

This research and development contract has been concerned with studies of the possible value of characteristic t-RNA breakdown products as markers for cancer. Methylated purines, pseudouridine, and beta-amino isobutyric acid have been studied as markers for tumors and as indicators of effective chemotherapy. Recently an instance was found of two abnormal methylated bases in the phenylalanine t-RNA from two tumors, where host normal tissue had conventional t-RNA. Because of this contractor's increasing basic research interest, this contract will be phased out June 1978 in favor of future support from grants.

COMMUNITY BLOOD AND PLASMA SERVICE (N01-CM4-2150)

The purpose of this contract is to provide quadruplet units of HL-A typed platelets to patients in the NIH. The use of HL-A matched platelets decreases the risk of sensitization or production of antibodies to white cells and platelets in patients who require long-term hematologic support.

CONTROL DATA CORPORATION (N01-CM6-7107)

The purpose of this contract is to provide data management and processing which enables simple and rapid retrieval of clinical information related to the patient data base provided by the participating group of contractors, the Brain Tumor Study Group, who enter patients on study according to specified protocols for treatment of brain tumors.

CORDOVA CHEMICAL COMPANY (N01-CM8-7201)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

DOW CHEMICAL COMPANY (N01-CM2-3712)

This service-type contract is concerned primarily with the reproductive studies of three antineoplastic agents per year in accordance with the FDA Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use, dated January 1966. These studies are divided into three phases: 1) Phase I, general reproductive performance and fertility utilizing mice or rats of both sexes; 2) Phase II, teratology studies using mice and/or rats and rabbits; and 3) Phase III, perinatal studies employing mice and/or rats. This contract has been extended without additional funds until September 1978 to permit completion of all studies and preparation of final reports.

DUKE UNIVERSITY (N01-CM6-7010)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. In addition, this contractor supplies the neuropathology reference expertise for the entire Brain Tumor Study Group. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

ELKINS-SINN, INC. (N01-CM6-7103)

The objectives of this contract are to provide facilities and capabilities for the development and production of parenteral investigational dosage forms for the Division of Cancer Treatment. The contractor is responsible for conformity to Good Manufacturing Practices and is responsible for completing all required analytical testing on each product prepared. All products are packaged, labelled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01-CM1-0056)

The principal objective of this project is to study the cell cycle phase specificity of clinical drugs and drugs in development towards clinical trials. Drugs are evaluated for this ability to selectively inhibit progress through G_1, S, G_2 and M-phase of their proliferative cycle and for their selective cytocidal effects in these phases as well. Both synchronized and

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01-CM1-0056) (CONTINUED)

exponentially growing mammalian cell culture systems are used. The importance of such studies stems from the need to identify and develop drugs which may be specifically cytotoxic to non-proliferating but viable and clonogenic cells. The *in vitro* methodologies used were developed by the Contractor.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01-CM4-0102)

This agreement furnishes instrumental and biological support to progressively adapt a prototype cell microfluorometer and computer data analysis system to human tumor cell characterization studies and drug evaluation projects planned by the National Cancer Institute. Engineering support has accomplished the design, testing and delivery of a multiparameter cell flow microfluorometer and sorting device coupled with a PDP-11/40 computer system. This FMF system is receiving progressive improvements through volume sensing devices, new software development, and special maintenance as a result of developing DCT needs. Biological investigations are concerned with FMF characterization of tumor growth as a function of tumor age or size.

FARMITALIA (N01-CM5-7014)

The primary goal of this contract is modification of adriamycin and daunomycin in both the aglycone and the sugar moiety to develop analogs with decreased cardiotoxicity and myelosuppression and enhanced antitumor activity. New analogs with modifications in the aglycone side chain should not interfere with intercalation because atoms in the side chain are outside the DNA helix. It is expected that modification in the sugar will slow enzymatic hydrolysis of the glycosidic linkage. The contract will terminate this year.

FARMITALIA (N01-CM5-7036)

This contract has been concerned with the design and synthesis of new and novel second generation aglycones related to Adriamycinone and Daunomycinone with structural modifications designed to reduce cardiotoxicity, broaden the spectrum of activity and improve the therapeutic ratio. Chemical modification is directed toward developing aglycones which influence the binding of tumor cells to DNA and change the rate of cellular uptake. Structural modification is also directed toward increasing the lipophilicity and thereby alter the distribution of the drug. In addition to the synthesis of aglycones, the contractor also couples specified aglycones with selected sugar moieties. The contract is scheduled to terminate this year.

FLOW LABORATORIES, INC. (N01-CM5-0571)

This contract furnishes approximately 312,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

FLOW LABORATORIES, INC. (N01-CM6-7088)

This service contract provides the Division of Cancer Treatment with storage and distribution capabilities for the large volume of investigational and commercial drugs used in clinical trials. Approved orders for clinical drugs are packaged and shipped to destinations around the world. The contractor also provides a computerized inventory management system. This assures the proper rotation of stock, an adequate lead time to obtain new supplies of drugs, and the prompt removal of expired materials.

FRANKLIN INSTITUTE RESEARCH LABORATORIES (N01-CM7-7092)

This contract provides for the preparation, printing and distribution of Cancer Therapy Abstracts, Volumes 18, 19, and 20 for the years 1977 through 1979. Computer tapes of the abstracts are also supplied for use in the ICRDB data base, Cancerline. The price of the annual subscriptions pays the costs of printing and mailing by the Contractor. This current awareness journal, published monthly, supplies abstracts and annotations of the world's literature pertinent to the treatment of cancer. Hard copy of the articles cited in the journal are also received from the Contractor.

FRED HUTCHINSON CANCER RESEARCH CENTER (N01-CM7-7148)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage I tumors are randomized (double blind trial) to intrapleural BCG + INH x 12 weeks versus placebo intrapleural BCG + placebo INH x 12 weeks. Resectable Stage II and III epidermoid carcinoma is randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

FRONTIER SCIENCES ASSOCIATION (N01-CM7-7136)

Now performing only phase-out statistical support for the Gastrointestinal Tumor Study Group. Statistical support for GITSG is currently undergoing recompetition. Negotiations are presently underway.

GEORGETOWN UNIVERSITY (N01-CM3-3745)

Recompeted under RFP-CM8-7194 in February 1978. Contract to be awarded again to Georgetown to support Extramural Clinical Trials Operations Office (ECTO) under Ms. Grifone. Presently this office acts as randomization center for the Lung Cancer Study Group and the Head and Neck Contract Program. Also coordinates all correspondence with the above two groups in addition to the Gastrointestinal Tumor Study Group. This contract expired April 1978.

GEORGETOWN UNIVERSITY (N01-CM4-3732)

This contract has as its main objective the production of novel actinomycins by using biotransformation and co-metabolism fermentation techniques. These novel actinomycins will be produced in pure form in 100-500 mg. quantities and made available for broad-tumor screening. The use of many different compounds as substrates will be thoroughly explored. This project will expire this year and will not be renewed unless some of the compounds show marked superiority in animal antineoplastic tests to Actinomycin D, or show a more favorable therapeutic index.

GEORGETOWN UNIVERSITY (N01-CM6-7094)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

GEORGETOWN UNIVERSITY (N01-CM6-7110)

This contract supports a comprehensive program for research in treatment and care of patients with gastrointestinal cancer. The program includes a fully staffed and functioning GI unit, with participation by the surgery, radiology, clinical, pathology, and medical departments. Specifically, the GI Research Program includes studies in: early detection, staging, new and established anticancer agents, combinations and multidisciplinary approaches, pharmacology, evaluation of markers, and professional training. A new major effort is devoted to the study of implications of nutritional deficiencies and symptomatic manifestations of malignancies, and efforts to correct them. Specific areas of investigation being pursued include evaluation of neutron therapy in pancreatic cancer, development of drug regimens in gastric cancer, introduction and study of a new nitrosourea targeted towards gastrointestinal cancer, and evaluation of effect of plasmapheresis on tumor growth.

GEORGETOWN UNIVERSITY (N01-CM8-7194)

Contract to Georgetown to support Extramural Clinical Trials Operations Office (ECTO) under Ms. Grifone. Presently this office acts as randomization center for the Lung Cancer Study Group and the Head and Neck Contract Program.

GEORGETOWN UNIVERSITY (N01-CM8-7194) (CONTINUED)

Also coordinates all correspondence with the above two groups in addition to the Gastrointestinal Tumor Study Group.

GRACE, W. R. (N01-CM6-7074)

This contract provides for the fractionation of confirmed active W. R. Grace fermentations. These fermentations were from various unique industrial waste substrates and presumptive chromatography indicates they are novel antineoplastic agents. Enough of the active material will be isolated to be thoroughly evaluated. Six compounds were isolated this year in sufficient amounts to be tested *in vivo*. Already two show interesting activity. An additional 28 culture fermentations are undergoing chemical fractionation to isolate the active antineoplastic agents.

HARLAN INDUSTRIES, INC. (N01-CM5-0591)

This contract furnishes approximately 312,000 six-week-old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or production centers.

HARLAN INDUSTRIES, INC. (N01-CM6-7079)

This contract provides for the maintenance of a rodent production center. This center produces athymic (nude) mice for research and testing laboratory programs. All activities are performed in biocontainment environments.

HARLAN INDUSTRIES, INC. (N01-CM7-7168)

This primary genetic center establishes associated flora foundation colonies from which pedigreed expansion colonies are propagated in barrier rooms.

HARVARD COLLEGE (N01-CM5-3825)

This project is based upon the exploration of the possibility that disarming malignant cells, by increasing their immunogenicity by altering their cell surface polysaccharide composition, may be an effective chemotherapeutic approach. A detailed study of the effects of naturally occurring sugars and of sugar analogs on the oncogenicity of tumor cells and on the composition of their membrane glycoproteins, is being performed.

HAWAII, UNIVERSITY OF (N01-CM3-3747)

This contract provides for the collection of 3 to 5 lb. plant samples from the indigenous and exotic higher plants of the Hawaiian Islands and Pacific area and the recollection of larger samples of those that are reproducibly active in the Developmental Therapeutics Program tests. Plants so obtained are shipped to the extraction contractor, WARF Institute, Inc., and the extracts tested at one of our contract screeners. Larger recollections are shipped to the individual fractionators as assigned by the Developmental Therapeutics Program.

HAZLETON LABORATORIES, INC. (N01-CM3-3708)

The objectives of this contract are as follows: (a) to obtain comparative data on the response of primates to known rodent carcinogens and to materials suspected of being carcinogenic in man; (b) to evaluate the long-term effects of antineoplastic and immunosuppressive agents which are being used clinically; (c) to obtain model tumor systems in primates for evaluation of new and established antitumor agents; (d) to make available normal and tumor-bearing animals for pharmacologic, toxicologic, biochemical, and immunological studies; (e) to develop biological markers and diagnostic tests for detecting pre-neoplastic changes as well as frank neoplasia; (f) to develop models for prevention of carcinogenesis, especially for protecting against potent primate carcinogens and against the carcinogenic effects of clinically useful antitumor agents; and (g) to maintain a breeding colony of various species of primates so that these primates may be readily available for use. All work is carried out in close cooperation with the Laboratory of Chemical Pharmacology, NCI.

HAZLETON LABORATORIES, INC. (N01-CM5-7007)

Synthetic materials, crude materials of natural product origin, and fractions of previously confirmed active crude natural products are tested under this service contract. This testing is conducted according to Drug Evaluation Branch protocols at a rate of 25,000 L1210 equivalents per year. New synthetic materials and crude natural products are pre-screened in the P388 lymphocytic leukemia. Upon request, confirmed active materials may be tested in the tumor panel exclusive of the xenografts and mammary tumor systems. Research and development work is conducted upon the request of members of the Drug Evaluation Branch.

HAZLETON LABORATORIES, INC. (N01-CM6-0125)

This contract provides 200 beagle hounds for toxicological assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

HAZLETON LABORATORIES, INC. (N01-CM6-7080)

This contract supplies rapid and accurate clinical microbiologic information in support of patients in the Baltimore Cancer Research Program who receive cancer chemotherapy. It also provides patient microbiological surveillance cultures and environmental studies to afford a protected environment for patients in the Baltimore Cancer Research Program and the Pediatric Oncology Branch at NIH. Preliminary data are teletyped to the Cancer Centers for use in patient care, and the final data are permanently stored in a computer. Retrieval of this information by computer techniques affords the data base for clinicians and research investigators. Microbiological report summaries are placed in the patients' permanent medical record. This contract terminated April 1978.

HAZLETON LABORATORIES, INC. (N01-CM7-7144)

This is a short-term contract in which a DNBA mammary tumor will be developed in the rat and used for the study of hormone sensitivity and chemotherapy interaction. At the present time, there is an ongoing clinical trial in patients with metastatic breast cancer in which the possibility that stimulation of breast cancer cell growth with hormones will render tumor cells more susceptible to the cytotoxic action of chemotherapy. This clinical model will be verified by experiments with the DNBA rat mammary tumor. The study terminated in March 1978.

HAZLETON LABORATORIES, INC. (N01-CM8-7156)

This contract provides for the supply of dogs for leukocyte transfusion and bone marrow autograft experiments. The specific experiments underway seek dose-response relationships in leukocyte transfusion of leukopenic dogs with pseudomonas sepsis, and examine the bone marrow stem cell dose, using CFU-C, to reconstitute hemopoiesis after marrow ablative chemo- or radiotherapy.

HEALTH RESEARCH, INC. (N01-CM4-3782)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease.

HEALTH RESEARCH, INC. (N01-CM4-3794)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

HEALTH RESEARCH, INC. (N01-CM5-3834)

This contract is designed to support prospective randomized, controlled studies using combination chemotherapy in the treatment of breast cancer. This contract expired April 1978.

HEALTH RESEARCH, INC. (N01-CM5-7034)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

HEALTH RESEARCH, INC. (N01-CM5-7039)

The objective of this contract is to establish in vivo and in vitro immunoassay systems to be used to test the selective effects that various anticancer drugs may have on the immune response. The studies include evaluation of the immunosuppressive activity of the antineoplastic agents in order to aid in the selection of candidate drugs; evaluation of the immunostimulating properties of various agents for possible application in cancer therapy; and determination of the effect of drugs on the antigenic properties of normal and tumor tissues in order to assess any influence on chemotherapeutic response.

HEALTH RESEARCH, INC. (N01-CM6-7117)

A collaborative group whose main thrust is to study various post-surgical adjuvants in early epithelial tumors of the ovary. A joint protocol for good risk Stage I (i.e., FIGO Stage IAi and IBi) compares observation to L-PAM post-operatively. A secondary study involves a histo-pathologic study of the incidence of unsuspected pelvic and/or paraortic node metastases in this patient population. A further joint protocol for other early disease (i.e., FIGO Stage IAii, IBii, IC, and II) separates patients according to presence or absence of macroscopic residual disease post-operatively. Patients with macroscopic residual disease are randomized to L-PAM versus L-PAM + pelvic radiotherapy. Patients without macroscopic residual disease are randomized to L-PAM versus intraperitoneal instillation of 15 mc of radioactive chromic phosphate.

Additionally, the investigators are evaluating chemotherapeutic regimens in Stage III & IV disease, their most recent protocol being high-dose MTX + cytoxan versus 5-FU + cytoxan. Related investigations are concentrating on ovarian carcinoma antigens and other markers.

HEALTH RESEARCH, INC. (N01-CM6-7118)

The purpose of this contract is to investigate various aspects of the pharmacologic, toxicologic and therapeutic properties of antitumor agents (methotrexate, cytosine arabinoside, adriamycin, cis-dichlorodiammine platinum, melphalan and vincristine) encapsulated in liposomes. The physiological disposition of liposomes prior to drug encapsulation is being studied in both normal and tumor-bearing animals given liposomes by both iv and ip routes, with particular emphasis on the effect of liposome size, charge and chemical composition. The goals of the *in vivo* studies on liposome-encapsulated antitumor agents are: to establish the optimal conditions for drug incorporation into liposomes; to compare the physiologic disposition of the liposome-encapsulated antitumor agents with that of the non-encapsulated drugs in tumor-bearing animals treated by iv and ip routes; to determine the *in vivo* antitumor activity of liposome-encapsulated antitumor agents and compare it to that of the non-encapsulated drugs; and to evaluate the acute toxicity of the liposome-encapsulated agents in normal and tumor-bearing animals.

HEALTH RESEARCH, INC. (N01-CM7-7101)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The Contractor carries out the service by: (1) performing iso-antigenic typing tests of tumors used in the various screening tests, enabling the Developmental Therapeutics Program to maintain and distribute tumors identical to the prototype; (2) developing techniques and material for antigenic typing of tumors new to the screening program; (3) determining strain specificity of tumors originating in the inbred mouse; and (4) performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic production centers.

HEM RESEARCH, INC. (N01-CM6-7066)

The main objectives of this contract are to (1) provide single cell clones of primate type-C virus infected cell cultures; (2) characterize the cell and viral nucleic acids of these clones; and (3) isolate and characterize nucleic acids from human neoplastic tissues with respect to type-C virus related molecules.

HOUSTON, UNIVERSITY OF (N01-CM8-7209)

The objectives of this contract are the collection, preparation of extracts and isolation and identification of antineoplastic agents from marine organisms. A large number of active extracts have been produced and 20-30 of the most active organisms have been recollected in large amounts and are undergoing fractionation studies.

IIT RESEARCH INSTITUTE (N01-CM4-3755)

This contract provides assistance to the Drug Evaluation Branch staff in monitoring and evaluation of test data and the follow-up of materials demonstrating activity in the primary in vitro and in vivo screens. The Contractor participates with staff in the expediting and scheduling of testing and the evaluation of data on those materials which are recommended for testing in the panel of antitumor test systems. Design of data files used as management tools by Developmental Therapeutics Program staff has been provided and the coordination of the data input to these files continues to be provided by the Contractor.

IIT RESEARCH INSTITUTE (N01-CM5-3829)

The Contractor performs bacteriologic diagnostic services with emphasis upon the detection of Salmonella spp. and Pseudomonas spp. in program rodents and other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. The scope of performance of this contract was changed recently to the extent that they are now monitoring animals leaving the genetic and rodent production centers primarily for the presence of ecto and endoparasites. They also forward blood serum samples to the Microbiological Associates where these same animals are monitored for virus diseases. The results of these monitoring studies will be utilized to upgrade the quality of animals being sent to the laboratories. Diagnostic procedures are also carried out at this facility when disease problems occur in the various animal breeding colonies or in the laboratories using these animals. This contract terminated November 1977.

IIT RESEARCH INSTITUTE (N01-CM5-7004)

This contract provides for the in vivo testing of synthetic materials and natural products. Screening is being conducted according to Drug Evaluation Branch protocols at a level of approximately 50,000 L1210 equivalent tests per year. New natural products and synthetics are tested in the P388 leukemia in the mouse host. Tumor panel testing is conducted in all panel systems including the xenografts. Methodology and development are carried out only at the direction of the Project Officer and during this report period have been devoted to the improvement of tumor panel test systems.

IIT RESEARCH INSTITUTE (N01-CM6-3832)

This contract provides cytotoxicity screening in KB, P388, and L1210 cell cultures. Standardized assays are performed in accordance with procedures described by Division of Cancer Treatment protocols primarily as an aid in isolation and purification of potential antitumor agents from natural plant or animal sources. Selected synthetic materials are also screened as required by the Program. Contractor also participates in methodology studies to improve existing methods or to develop new screens.

ILLINOIS, UNIVERSITY OF (N01-CM6-7090)

This contract provides for the fractionation of confirmed active plant extracts and fermentation products in order to isolate in a pure state and identify the active compound(s). Plant materials used in this work are obtained, for the most part, through the United States Department of Agriculture.

INDIAN CANCER RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-010-1)

This PL-480 Agreement is for the collection and identification of natural products indigenous to India, the preparation of crude extracts from these materials, and the testing *in vivo* of these extracts as well as synthetic materials synthesized in India.

For this purpose, an *in vivo* screening laboratory is maintained in Bombay, India, together with an animal breeding colony to supply the necessary host animals. Currently, the P388 lymphoid leukemia is utilized with testing conducted as per current Developmental Therapeutics Program protocols. The P388 tumor system is employed for the testing of natural products, fractions, and synthetics.

INDIANA UNIVERSITY FOUNDATION (N01-CM6-7057)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

INFORMATION MANAGEMENT SERVICES, INC. (N01-CP7-1010)

An interdivisional transfer of funds to DCCP, Biometry Branch, to support statistical services for the Lung Cancer Study Group headed by Dr. Mitchell Gail. Monies support extramurally contracted data management with IMS.

INFORMATION PLANNING ASSOCIATES, INC. (N01-CM7-7104)

Information Planning Associates, Inc. provides technical assistance and support services in the area of investigational drug regulations. Information is gathered and assembled for the preparation of Investigational New Drug Applications (IND's). This includes screening information, animal toxicology, chemistry, bibliographic information, drug labeling and the clinical protocol. This information is submitted to the Food and Drug Administration and an IND is awarded. This Contractor then maintains the files by amending information as necessary. IPA is also involved with the preparation of IND annual reports, the establishment of drug master files, the distribution of clinical brochures, and the dissemination of adverse drug reaction information.

INSTITUT JULES BORDET (N01-CM5-3840)

The objective of this contract is the maintenance of a liaison office at the Institut Jules Bordet, Cancer Center of the University of Brussels, Belgium. The program is designed to foster close collaboration between European and United States investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy. In addition to the information activities, an animal tumor test is maintained for the more detailed evaluation and development of selected new compounds, in accordance with National Cancer Institute interests.

INSTITUT JULES BORDET (N01-CM5-7040)

This contract provides *in vivo* screening of new synthetic and natural product materials and the follow-up testing of those demonstrating activity in the primary screen. The primary screen is carried out in accordance with the Developmental Therapeutics Program protocols. New materials are tested initially against the P388 leukemia test system. Compounds of interest to the program are also tested in the panel of antitumor systems (exclusive of the xenografts and mammary tumor systems) as requested. Detailed testing, such as schedule dependency studies with compounds of potential clinical interest, is also performed. Due to Program budgetary restrictions, the level of testing was reduced from 25,000 to 15,000 L1210 equivalent tests per year, effective January 1978.

INSTITUTE OF CANCER RESEARCH (N01-CM4-3736)

This project, formerly carried out at the London laboratories (Chester Beatty Research Institute) of the Institute of Cancer Research, includes tasks conducted at both the London and Surrey, England laboratories. The relocation of some tasks to Surrey coincided with the completion of the new Marsden Hospital and new Institute laboratories. The principal facets of the project are: (1) validation of human tumor xenografts as models for cancer chemotherapy; (2) the use of human tumor xenografts for testing new compounds of interest to DCT, NCI; (3) associated clinical studies; and (4) the design and acquisition of new antitumor agents based on results of locally supported biochemical pharmacological research.

INSTITUTE OF CANCER RESEARCH (N01-CM7-7139)

The objectives of this contract are to design and synthesize organic heterocyclic compounds that, in combination with radiotherapy, will selectively sensitize hypoxic cells for irradiation. It is widely believed that the radio-resistance of hypoxic tumor cells is the limiting factor in the effectiveness of radiation in the treatment of solid tumors. The compounds designed to sensitize hypoxic cells should mimic the oxygen effect and be active only against hypoxic cells. The chemical radiosensitizers belong to the electron-affinic class where the sensitizing efficiency is a direct function of the electron-affinic characteristics of the molecule. The Contractor will synthesize nitroimidazoles with electrophilic groups. These compounds will be evaluated for electron-affinity, lipophibicity, sensitizing efficiency, and cytotoxicity.

INSTITUTO NAZIONALE DE LA CURA DEI TUMORI (N01-CM6-7115)

This contract represents one component of a consortium of clinical centers studying the classification of non-Hodgkin's lymphoma. Because of the plethora of pathologic classifications in this area, the objectives of this collaborative effort are to collect and analyze retrospectively over 1000 cases of non-Hodgkin's lymphoma and correlate the clinical features (and lymphoid markers where available) with various pathologic classifications, seeking the major histologic determinants of clinical behavior. This contract will be phased out in September 1978.

IOWA, UNIVERSITY OF (N01-CM4-3743)

The objectives of this contract are to develop, manufacture and package parenteral dosage forms of investigational drugs for clinical trial. The Contractor has produced primarily sterile freeze dried injectable products, infusion fluids and tablets under this contract. However, he has the capability to produce a wide variety of pharmaceutical dosage forms. The Contractor is responsible for completing required analytical and safety tests on each product, as well as monitoring the stability of the dosage form at recommended and elevated temperatures. All products are packaged, labelled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

IOWA, UNIVERSITY OF (N01-HB6-2973)

This contract is concerned with clinical studies to determine the efficacy or granulocyte transfusions and in what patient populations they are useful.

IOWA, UNIVERSITY OF (N01-CM7-7176)

The major objective of this fermentation contract is the preparation of novel antibiotics using the fermentation techniques of biotransformation and co-metabolism. This effort is to convert materials which have solubility, toxicity or marginal activity problems to more active antineoplastic agents. A multitude of unique organisms are isolated and used to obtain these microbial conversions in an effort to obtain antineoplastic agents. This year the biotransformation of five compounds was started. Thirty-four different metabolites were formed and are being isolated to determine their antineoplastic activity.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (N01-CM3-3714)

The major thrust of this contract has been the multidisciplinary approach to breast cancer at all stages. Completed studies in the very important follow-up phase include the "CMF combination chemotherapy versus placebo following radical mastectomy for Stage II carcinoma" and the "CMF twelve versus six months of treatment" study in the same stage. In locally inoperable breast cancer and in the metastatic disease this contract has led the way in exploring the usefulness of chemotherapy and in developing non-cross resistance combinations, respectively. Work is continuing with innovative protocols at all stages. In other disease areas this contract has participated within the Gastrointestinal Tumor Study Group in colon adjuvant studies and is developing new protocols to test concepts of late intensification in testicular, small cell of lung, diffuse histiocytic lymphoma, and breast cancer.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (N01-CM4-3726)

This contract represents utilization of a unique resource of both advanced and early patients with malignant melanoma for various controlled clinical trials. The Project Director is the Chairman of the International Group for the Clinical Study of Melanoma. There are 27 collaborating institutions including Eastern and Western Europe, Australia (Sydney), and South America (Montevideo). Investigators have surgical, pathologic, chemotherapeutic and immunotherapeutic expertise. A clinical trial to determine the value of adjuvant DTIC, BCG, DTIC + BCG or no further therapy following lymph node dissection in patients with nodal melanoma metastases or Stage I lesions of the trunk is currently in progress. The study will determine whether the above adjuvant therapies increase the disease free interval for those patients. In addition, a trial of DTIC, DTIC + BCG, or DTIC + C. parvum in advanced malignant melanoma is also in progress. This study will determine the response rates and duration of response for patients with metastatic melanoma.

JACKSON LABORATORY (N01-CM6-0176)

This service contract provides for the procurement of approximately 52,000 AKR inbred mice to screening contractors for tumor transplantation and as breeders for large-scale hybrid production colonies. Breeding animals are supplied by the Contractor. This contract terminated November 1977.

JAPANESE FOUNDATION FOR CANCER RESEARCH (N01-CM2-2054)

The objective of this contract is the maintenance of a liaison office at the Japanese Foundation for Cancer Research in Tokyo. The program is designed to foster close collaboration between Japanese and United States investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy. A small testing facility is also maintained for the screening and further evaluation of selected new compounds.

JOHNS HOPKINS UNIVERSITY (N01-CM4-3718)

This was an effort to exploit pulse-labeling of myeloma proteins using carbon-14 guanidino-labeled arginine to quantitate patient tumor burden. Techniques were developed in a murine plasmacytoma model and then applied to patients. The technique was proven practicable in man but its validity and clinical utility remain to be determined. This contract was phased out February 1978.

KANSAS, UNIVERSITY OF (N01-CM1-2184)

The principal objective of this contract is to collect pharmacokinetic data on new and established antitumor agents in patients undergoing treatment for malignant disease and to analyze these data for individual variability which can be correlated with clinical response or more other pharmacologic parameter. Methotrexate, 5-fluorouracil, and dianhydrogalactone are currently under study. This contract will be phased out June 1978.

KANSAS, UNIVERSITY OF (N01-CM2-3217)

This contract provides research capabilities in the area of formulation design and development. Formulation problems not ammenable to the usual solubilization and/or stabilization methods are investigated. The Contractor has particular expertise in application of molecular complexes and reversible derivatives to improve solubility. Pilot batch preparation and chemical evaluation of these novel formulations are carried out under this contract. Compounds presently under investigation include 6-selenoguanosine, hydrazinecarbothioamide, 2-[5-amino-4-methyl-1-isoquinolinyl) methylene]-(chemical name for Sartorelli's thiosemicarbazone) and cis-dichlorodiammine platinum.

KANSAS, UNIVERSITY OF, MEDICAL CENTER (N01-CM4-3802)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations of regimens mutually agreed upon. Phase I studies have been conducted on anguidine (NSC-141537), chlorozotocin (NSC-178248), and imidazole pyrazole (IMPY: NSC-51143) during the past year. Studies on bruceantin (NSC-165563), and ICRF-187 (NSC-169780) are being planned. This contract will be terminated September 1978.

KENTUCKY, UNIVERSITY OF (N01-CM6-7038)

This is a resource and developmental contract concerned with study of terminal deoxynucleotidyl transferase (TdT) in bone marrow and peripheral blood cells of patients as a marker for diagnosis and therapy evaluation in leukemia. A large number of patients with acute lymphocytic leukemia have shown consistent, abnormal elevation of TdT correlating with activity of this disease. The sensitivity of the marker in predicting relapse following therapy is being evaluated. Efforts are being made to develop a simple immunofluorescent antibody technique for detecting TdT and relating TdT to specific cell types and to surface markers and ultrastructure. This contract will expire June 1978.

KENTUCKY, UNIVERSITY OF (N01-CM6-7058)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

LABORATORY RESEARCH ENTERPRISES, INC. (N01-CM6-0124)

This contract provides 200 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

LABORATORY SUPPLY COMPANY, INC. (N01-CM5-0577)

This contract furnishes approximately 312,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

LABORATORY SUPPLY COMPANY, INC. (N01-CM5-3775)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigators. This contract also furnishes breeding animals for large-scale production colonies. All activities are performed in bio-containment environments.

LEEDS, UNIVERSITY OF (N01-CM4-3727)

The purpose of this contract is the investigation of the usefulness of various "markers" in the study of gastrointestinal cancers with special reference to the earlier detection of metastases and their pattern of change during the evolution of the disease. Phase II chemotherapy studies in advanced gastric and colorectal carcinomas are also being performed.

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (N01-CM7-7165)

This primary genetic center has as its objective the development of associated foundation colonies of inbred rodents. Pedigree animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of non-pathogenic organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to secondary genetic centers, which, in turn, produce breeding animals for large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small-scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies.

LITTON BIONETICS, INC. (N01-CM1-2340)

The purposes of this contract are the following: (1) to purify selected cellular and retroviral structural proteins and to analyze their primary structure and antigenic relatedness; (2) to purify and analyze selected cellular and viral DNA polymerases; (3) to prepare various viral nucleic acid probes from selected retroviruses and to analyze cellular nucleic acids for sequence homology; (4) to test cellular macromolecules and sub-cellular particles for possible virologic or tumorigenic biological activity; (5) to purify and analyze selected stimulators of normal and leukemic

LITTON BIONETICS, INC. (N01-CM1-2340) (CONTINUED)

leukocyte growth and differentiation *in vitro*; and (6) to study the genotypic and phenotypic elements involved in the disturbances of leukemic leukocytes growth and differentiation.

LITTON BIONETICS, INC. (N01-CM2-2062)

This contract has three main objectives: (1) to provide substantial quantities (400 grams per annum) of tissue cultured normal and malignant mammalian cells; (2) to receive, process, analyze, distribute and store up to 200 fresh human blood samples, principally from leukemic patients; and (3) to grow normal and leukemic human leukocytes in short- and long-term culture under conditions specified by the GPO. The accomplishment of the above tasks requires the maintenance of strict quality control procedures, of accessible inventories of certified cellular materials and of appropriate biohazard safeguards and the performance of selected analytical procedures, including cellular cytochemistry, cell pulse-labelling procedures, and DNA polymerase assays on culture media concentrates. This contract will terminate August 1978.

LITTON BIONETICS, INC. (N01-C07-5380)

The Government-owned, contractor-operated Frederick Cancer Research Center serves as a multi-faceted contract involving the following activities for the Division of Cancer Treatment:

Biologic Markers Laboratory

A program of active research and development of biologic markers as aids to effective cancer therapy has been established. The investigative program is supported by an analytical division and a human specimen repository. These provide rapid and comprehensive testing of newly generated or improved candidate cancer markers in comparison with older and presently employed markers. The repository specimens are acquired largely from protocol clinical investigations and are well documented. A fourth division of the Biologic Markers program deals with specialized and difficult problems in drug metabolism and analysis.

Chemotherapy Fermentation Laboratory

At the Frederick Cancer Research Center, the Division of Cancer Treatment has a fermentation pilot plant facility for the large-scale production and isolation of microbial products of interest to the program. Facilities are also available for the production of other natural products. This year kilogram quantities of one drug were obtained plus the production of interferon and several enzymes. A fermentation research laboratory has been completed and will be used to systematically evaluate various microbes' abilities to ferment unique substrates and produce novel compounds. The Division also participates in the operation of the Animal Farm and environmental control aspects of the program at FCRC.

LITTON BIONETICS, INC. (N01-CM5-3778)

The Contractor performs bacteriologic diagnostic services with emphasis upon the detection of Salmonella spp. and Pseudomonas spp. in program rodents and other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. Approximately 10,000 samples will be screened per year. The research portion of this contract (a minor component) is directed towards improvement in cultural techniques.

LITTON BIONETICS, INC. (N01-CM6-7067)

This contract supports the efforts of the Surgery Branch to monitor patients undergoing immunotherapy. The immunologic reactivity of these patients before, during, and after treatment with immune adjuvants requires storage and cryopreservation of lymphocytes and serum for subsequent testing. Because of the difficulties of day-to-day variability in many of the bioassays used to study the immune response, it is critical that these reagents be stored and cryopreserved in such a fashion as to allow simultaneous estimation of such assays as mitogen stimulation, immunoglobulin determinations, cellular cytotoxicity, and other tests of immunologic function. Further, the Contractor assists in the tracking and following of all patients on immunotherapy protocols for the purpose of correlating the clinical data with the immunologic findings.

LITTON BIONETICS, INC. (N01-CM7-7134)

The purposes of this contract are as follows: (1) to provide laboratory facilities for maintaining various experimental animals, including sub-human primates; (2) to provide biohazard facilities which permit in vitro and in vivo studies of primate retroviruses; (3) to provide all facilities essential to tumorigenic studies, including all necessary personnel and equipment for the procurement and analysis of blood and other body fluids and of surgical or post-mortem tissue samples; and (4) to prepare and analyze antisera to selected, purified cellular and viral proteins.

LITTON BIONETICS, INC. (N01-CM8-7169)

A contract has been awarded which will assist the intramural scientists in the Clinical Oncology Program in the storage and maintenance of laboratory animals. The facilities of the Clinical Center are extremely limited in the availability of space and personnel for laboratory animal handling, and this investigative resource is commonly available through a contract mechanism. The new contract combines the animal holding and transport needs of all branches in Clinical Oncology into one support contract. The Contractor will maintain, feed, and transport the animals but will not conduct research. Tumored animal models will also be provided.

MADRID, UNIVERSITY OF (N01-CM5-3792)

The objective of this contract is to evaluate the effects of antitumor drugs on the electron transport processes in aerobic energy metabolism. The systems used are isolated heart and liver mitochondria, and intact Ehrlich ascites cells, using O_2 electrode measurements of oxygen consumption as well as spectroscopic methods to determine the oxidation state of electron transport chromophores. In addition, the action of selected drugs on myocardial membrane Na-K dependent ATPase is being evaluated. As a result of work done under this contract, a new anti-cancer agent, quelamycin (a derivative of adriamycin with considerable less cardiotoxicity) was developed and is in clinical trial. This contract terminated March 1978.

MAKERERE UNIVERSITY COLLEGE COUNCIL (N01-CM7-1343)

This contract was developed to operate the Lymphoma Treatment Center and the Solid Tumor Center, collectively known as the Uganda Cancer Institute, to follow-up facilities for patients with Burkitt's lymphoma, Hodgkin's disease, and other neoplasms. It is continuing under the direction of Dr. Sebastian Kyalwazi and Dr. Charles Olweny. This contract has been phased back to a level commensurate with appropriate surveillance of patients treated in the past, but does not support newly-initiated clinical trials.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CM3-3720)

The primary purpose of this project is to establish a regional European contract as an extension of the Division of Cancer Treatment compound acquisition and screening program. The Contractor collects materials in Southern European countries and performs screening at the rate of approximately 7,500 L1210 equivalency tests per year. The P388 tumor system is utilized as a pre-screen and tumor panel systems include the B16 melanoma, L1210, Lewis lung and Colon 38.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CM5-3826)

This contract is for the purpose of development of immunochemotherapy studies on established or new experimental model systems. The studies include the development of new tumor-host systems in inbred as well as hybrid mice in various genetic combinations as well as studies of combined modalities of antineoplastic immunotherapy and chemotherapy.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CM6-7064)

This contract continues to investigate the relationship between drug kinetics and drug effect with the adriamycin analogue N-trifluoroacetyladriamycin-14-valerate (AD 32). Methods have been developed to measure AD 32 and its metabolites. Pharmacokinetic behavior is being monitored in blood and tissues.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CM6-7064) (CONTINUED)

Like adriamycin, AD 32 is both immunodepressive and myelosuppressive but qualitatively different in these respects from adriamycin. A major metabolite of AD 32, AD 41, exhibits *in vitro* cytotoxic activity similar to that of the parent compound.

MARSHALL RESEARCH ANIMALS, INC. (N01-CM6-0123)

This contract provides 200 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

MARYLAND, UNIVERSITY OF (N01-CM4-3748)

Under this service contract, the University of Maryland at Baltimore provides suitable facilities, administrative support, ancillary support services and personnel for the inpatient operation of the Baltimore Cancer Research Program, as well as space for activities such as Outpatient Department, Cell Component Therapy, Clinical Research Pharmacy, and laboratory efforts necessary for the conduct of the program.

MARYLAND, UNIVERSITY OF (N01-CM8-7223)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The initial protocol is being written and will study the toxic effects as well as the therapeutic benefits of intensive preoperative chemotherapy and maintenance chemotherapy when added to a standard regimen of surgery and postoperative radiotherapy. Controls will receive the standard surgery/radiotherapy regimen alone. Study sites will be limited to the anterior tongue, floor of the mouth and retromolar trigone/anterior tonsillar pillar areas in the oral cavity, and glottic larynx, supraglottic larynx and pyriform sinus in the laryngopharyngeal region. Only patients with Stage III and IV lesions will be studied, except for patients with Stage II pyriform sinus lesions.

MASON RESEARCH INSTITUTE (N01-CM5-7003)

This service contract is for the *in vivo* testing of synthetic and natural products which are submitted to the Developmental Therapeutics Program. The P388 tumor system is utilized for the pre-screen testing of these materials. In addition, testing is carried out on those compounds assigned to tumor panel testing in the B16 melanoma, Colon 26, Colon 38, Lewis lung, L1210, Colon xenografts CX-1 and CX-2, Lung xenograft LX-1 and Mammary

MASON RESEARCH INSTITUTE (N01-CM5-7003) (CONTINUED)

xenograft MX-1. Testing is carried out at a level of approximately 37,000 L1210 equivalent tests per year. Schedule dependency studies to determine optimal dose, schedule and route of administration, combination studies and special studies on other tumor test systems are conducted as directed by Drug Evaluation Branch staff.

MASON RESEARCH INSTITUTE (N01-CM5-7030)

New experimental solid tumor models will be developed and evaluated in order to improve the predictive value of tumors in selecting drugs which will be active in human solid tumors. As a result of these studies it has been recommended that the M5076 ovarian carcinoma be added to the tumor panel used in selecting candidate drugs for eventual clinical trial. In addition, emphasis will be placed on establishing human tumor xenografts in athymic nude mice, studying the biology of xenografts, and developing methods for assessing effects of drugs and other treatment modalities in this tumor model.

MASON RESEARCH INSTITUTE (N01-CM6-7011)

This contract supports the Clinical Oncology Program of the Division of Cancer Treatment with computer programming expertise for the development of clinical information systems and with data technician services for the maintenance and utilization of these systems. A wide variety of systems have been developed and are maintained for the clinical Branches of the Division of Cancer Treatment.

MASON RESEARCH INSTITUTE (N01-CM8-7164)

The frozen tumor bank maintains and ships experimental tumors to screening laboratories and other qualified investigators throughout the United States and abroad. Tumors are monitored on a scheduled basis for viral and bacterial contaminants.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (N01-CM4-3712)

Bleomycin is a clinically effective antitumor antibiotic. The basic objective of this contract is to investigate whether the main activity of this antibiotic can be ascribed to some portion of the molecule. The plan is to synthesize partial structures and analogs structurally related to bleomycin and by screening find that moiety which possesses the greatest activity with minimal toxicity. Such determination can be valuable because it provides ideas for the synthesis of more effective analogs. This contract will terminate this year.

MAYO FOUNDATION (N01-CM0-2066)

The purpose of this contract is the investigation of new drugs in the treatment of advanced gastrointestinal tumors as a cooperative venture between the Mayo Foundation and the National Cancer Institute. The objective is to have each drug evaluated in a minimum of 20 cases of adenocarcinoma of the colon to meet the requirements of the Division of Cancer Treatment program linear array. Provision has been made for reporting of data on each trial in a manner which is consistent with the data retrieval currently employed intramurally at the National Cancer Institute. This will enable both direct comparisons and possible integration of data on new drugs from both of these sources. This contract will terminate September 1978.

MAYO FOUNDATION (N01-CM4-3783)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease.

MAYO FOUNDATION (N01-CM4-3796)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

MAYO FOUNDATION (N01-CM5-3772)

This contract is designed to support prospective randomized, controlled studies using combination chemotherapy in the treatment of breast cancer. This contract will expire June 1978.

MAYO FOUNDATION (N01-CM5-3838)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies have been conducted on hycanthone (NSC-142982), chlorozotocin (NSC-178248), indicine-N-oxide (NSC-132319) and alanosine (NSC-153353) during the past year. This contract will terminate September 1978.

MAYO FOUNDATION (N01-CM5-7033)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

MAYO FOUNDATION (N01-CM5-7044)

This contract is designed to support Phase II-III studies in solid tumors, by means of prospective randomized, controlled clinical trials involving the use of combined modalities.

MAYO FOUNDATION (N01-CM6-7123)

A collaborative group whose main thrust is to study various post-surgical adjuvants in early epithelial tumors of the ovary. A joint protocol for good risk Stage I (i.e., FIGO Stage IAi and IBi) compares observations to L-PAM post-operatively. A secondary study involves a histo-pathologic study of the incidence of unsuspected pelvic and/or paraortic node metastases in this patient population. A further joint protocol for other early disease (i.e., FIGO Stage IAii, IBii, IC, and II) separates patients according to presence or absence of macroscopic residual disease post-operatively. Patients with macroscopic residual disease are randomized to L-PAM versus L-PAM + pelvic radiotherapy. Patients with macroscopic residual disease are randomized to L-PAM versus intraperitoneal instillation of 15 mc of radioactive chromic phosphate.

The Mayo Clinic investigators are also evaluating, in a randomized study of advanced disease, the use of cytoxan versus cytoxan + cis-platinum, having recently completed a trial of cytoxan versus cytoxan + adriamycin.

MAYO FOUNDATION (N01-CM7-7150)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage I tumors are randomized (double blind trial) to intrapleural BCG + INH x 12 weeks versus placebo intrapleural BCG + placebo INH x 12 weeks. Resectable Stage II and III epidermoid carcinomas are randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM4-3804)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies have been conducted on chlorozotocin (NSC-178248), gallium nitrate (NSC-15200), rubidazone (NSC-164011), m-AMSA (NSC-249992), PALA (NSC-224131), and the combination of 5-fluorouracil and thymidine, during the past year. This contract will terminate September 1978.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM5-3844)

This contract is designed to support Phase II-III studies in solid tumors by means of prospective randomized, controlled clinical trials involving the use of combined modalities.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM5-7019)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM5-7043)

This contract is for Phase II/III studies to detect useful therapeutic effects of new drugs alone and in various combinations in various solid tumors. This contract has completed 30 months of a 5 year contract. Since the annual review in February 1977, 8 new protocols have been approved for inclusion under the terms of the contract, increasing the number of current studies to 18. Patient entry in this period is 533 bringing the total patient entry to 1663 in 27 protocols. Unique information has been gained from this contract in the treatment of germ cell tumors, epidermoid tumors, head and neck cancer, breast cancer, oat cell cancer, and bladder cancer.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM8-7224)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The initial protocol is being written and will study the toxic effects as well as the therapeutic benefits of intensive preoperative chemotherapy and maintenance chemotherapy when added to a standard regimen of surgery and postoperative radiotherapy. Controls will receive the standard surgery/radiotherapy regimen alone. Study sites will be limited to the anterior tongue, floor of the mouth, and retromolar trigone/anterior tonsillar pillar areas in the oral cavity, and glottic larynx, supraglottic larynx and pyriform sinus in the laryngopharyngeal region. Only patients with Stage III and IV lesions will be studied, except for patients with Stage II pyriform sinus lesions.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CP6-5829)

As part of a multi-institutional cooperative effort, the effectiveness of routine nutritional practice will be compared with that of total parenteral nutrition via central-line hyperalimentation. Eight pediatric patients with malignant tumors of the abdomen and/or pelvis requiring radiotherapy and intensive courses of multiple chemotherapeutic agents will be studied. Comparisons will be made with respect to: (a) tolerance to therapeutic schedule; (b) therapeutic response to combined chemotherapy and radiotherapy; (c) effectiveness of having the bowel "at rest" with respect to effects of therapy on gastrointestinal function; (d) nutritional status of the patient; and (e) complications during therapy. It is hypothesized that maintaining good nutritional status through total parenteral nutrition during combined radiotherapy to the abdomen and intensive chemotherapy, will result in fewer complications, decreased hospitalization time, and better therapeutic response. This hypothesis will be tested by a prospective randomized study comparing the patients maintained by total parenteral nutrition with those on routine dietary intake.

MIAMI, UNIVERSITY OF (N01-CM4-3800)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

MIAMI, UNIVERSITY OF (N01-CM5-7041)

The objective of this contract is to measure the immunosuppressive effects of drugs in order to determine how tissue and dose variables of treatment influence the depth and duration of suppression. These studies employ existing *in vitro* systems to determine the effect of the various antineoplastic agents on humoral and cell-mediated immune response.

MIAMI, UNIVERSITY OF (N01-CM6-7077)

The *in vitro* testing of crude materials of natural product origin, fractions of confirmed natural products, and of synthetic materials submitted in too small quantity for *in vivo* testing is conducted by this service contract laboratory. Routinely, all materials are tested in the 9KB tumor system with testing in the P388 and/or L1210 system conducted on those crude natural

MIAMI, UNIVERSITY OF (N01-CM6-7077) (CONTINUED)

products that were active *in vivo* and not cytotoxic in 9KB. These tests are conducted according to protocols distributed by the Drug Evaluation Branch at a level of 15,000 9KB test equivalents per year. At the request of the Project Officer, research and development testing is conducted.

MIAMI, UNIVERSITY OF (N01-CM6-7093)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

MICHIGAN, UNIVERSITY OF (N01-CM8-7225)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The initial protocol is being written and will study the toxic effects as well as the therapeutic benefits of intensive preoperative chemotherapy and maintenance chemotherapy when added to a standard regimen of surgery and postoperative radiotherapy. Controls will receive the standard surgery/radiotherapy regimen alone. Study sites will be limited to the anterior tongue, floor of the mouth and retromolar trigone/anterior tonsillar pillar areas in the oral cavity, and glottic larynx, supraglottic larynx and pyriform sinus in the laryngopharyngeal region. Only patients with Stage III and IV lesions will be studied, except for patients with Stage II pyriform sinus lesions.

MICHIGAN CANCER FOUNDATION (N01-CM5-3789)

The objective of this project is to characterize the range of molecular biological effects of antitumor drugs. In addition to effects on nucleic acid synthesis, the effects of new drugs on DNA structure, cell membrane permeability, cell surface electrical charge, cell surface adhesive properties and enzymes involved in the synthesis of cell surface glycoproteins will be studied. This contract terminated March 1978.

MICHIGAN DEPARTMENT OF PUBLIC HEALTH (N01-CM3-3702)

This contract provides assay services, utilizing a variety of cell culture methods, as aids in the purification of potential antitumor agents from fermentation broths. The methods employed include paper chromatography and paper electrophoresis in conjunction with cell culture agar diffusion bioautography, as well as growth inhibition assays, both in stationary and suspension cultures. The related fermentation and isolation program services by this assay contract are financed by the Michigan Department of Public Health.

MICROBIAL CHEMISTRY RESEARCH FOUNDATION (N01-CM5-7009)

The major objective of this contract is the preparation of fermentations of marine, psychrophilic and thermophilic organisms. These fermentations are screened against various enzyme and other biochemical screens. Active products are isolated in sufficient quantities to be evaluated at the National Cancer Institute. In addition, various immunogenic tests have been developed to evaluate the organisms and their metabolites as potential immunological stimulators specific for cancers.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM4-3793)

This contract provides the National Cancer Institute with services for the storage and distribution of bulk chemicals and drugs. Samples are packaged and shipped to various domestic and foreign research laboratories. This contract provides for the storage, weighing, packing and shipping of chemical samples and the maintenance of accurate inventory records.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM5-3818)

This rodent production center supplies inbred rodents for tumor transplantation, for hybrid production, and for compound evaluation studies. Animals are supplied from a colony of two strains of rodents.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM6-3810)

This contract resource assists in the measurement of leukocyte compatibility in clinical transfusions of leukopenic patients. The Contractor performs several leukoagglutinin assays and lymphocyte cross-match studies in order to insure safe and reliable blood component transfusions. These tests are used in the daily management of the NCI leukopheresis program. In addition, the Contractor operates a computerized serum storage bank for all patients at NCI which has accessioned over 20,000 samples.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM6-7069)

This contract has as its major purpose serological diagnosis for the presence or absence of infectious mouse ectromelia, LCM, polyoma, M. hepatitis, M. adenovirus, PVM, Sendai, Theiler's (GDVII), K, Rheo 3, Toolan's H-1, and the Rauscher and Moloney viruses. The Contractor also carries out ancillary research concerned with the effects of viruses, such as vaccinia and polyoma, on the growth of tumors; interaction of polyoma virus with tumors; antibody-producing capacity of tumor-bearing mice and the effect of the route of injection on antibody levels; and a comparison of the sensitivity of caesarian-derived and other mice to polyoma virus. This activity furnishes information on the effects of "passenger" agents on the growth of tumors used in the screen and on their effects on drug action. During a contract period

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM6-7069) (CONTINUED)

of one year, this laboratory performs approximately 65,000 rodent serum and 6,000 tumor fragment diagnostic tests. The Contractor also prepares large quantities of vaccinia virus used for immunizing mice against infectious ectromelia.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM7-7131)

The objective of this contract is to provide diagnostic viral studies, cultural, seriological, and EM, on samples from patients who participate in the National Cancer Institute's chemotherapy program in the Baltimore Cancer Research Program, and at the National Cancer Institute, Bethesda, Maryland. The results will not only aid the clinician in selecting optimal supportive care, but may also confirm the cause of obscure symptomatology and help clarify possible virus-bacterium interrelationships in immunosuppressed cancer patients. This contract will terminate August 1978.

MIDWEST RESEARCH INSTITUTE (N01-CM3-3722)

Midwest Research Institute is one of the two facilities where the chemical analysis of experimental medicinal compounds and their clinical formulations are performed for the Developmental Therapeutics Program. The Contractor determines the identity, the purity, the solubilities, the stability, and physiochemical constants such as pKa and log P of these materials. The Contractor develops procedures for analysis when standard procedures are not available. The procedures employed include elemental analysis, chromatography, spectrometry and other physiochemical methods appropriate to the situation. This contract provides information as a guidance to the effective use, formulation and storage of the materials; provides quality assurance for the release of the materials for investigational use; and provides the necessary analytical data for filing with the Food and Drug Administration.

MIDWEST RESEARCH INSTITUTE (N01-CM3-3743)

The Contractor is synthesizing unique compounds for cancer chemotherapy studies. This includes design and synthesis of analogs of active structures in the areas of alkaloids, antibiotics, quinones, receptor site-oriented compounds, protease inhibitors, and other areas which may be of value in cancer chemotherapy as potential anticancer agents. Such multiple faceted research is needed to permit maximum response to changes in program interests. This contract will be phased out in 1978.

MILES LABORATORIES (N01-CM4-3709)

This is a no-cost contract concerned with the marketing of a clinically useful drug, DTIC. The Contractor summarized the clinical and other data, prepared and filed the New Drug Application (NDA) with the Food and Drug Administration, and prosecuted the NDA. The NDA has been approved, and the drug is currently on the market in the United States and in several other countries.

MINNESOTA, UNIVERSITY OF (N01-CM4-3788)

The major emphasis of this project is directed toward determining the cell uptake transport processes of selected antitumor drugs. In addition, the effects of membrane active, natural substances on the uptake and effectiveness of antitumor drugs are being evaluated.

MINNESOTA, UNIVERSITY OF (N01-CM6-7113)

This contract represents one component of a consortium of clinical centers studying the classification of non-Hodgkin's lymphoma. Because of the plethora of pathologic classifications in this area, the objectives of this collaborative effort are to collect and analyze retrospectively over 1,000 cases of non-Hodgkin's lymphoma and correlate the clinical features (and lymphoid markers where available) with various pathologic classifications, seeking the major histologic determinants of clinical behavior. This contract will be phased out September 1978.

MINNESOTA, UNIVERSITY OF (N01-HB6-2972)

This contract is concerned with clinical studies to determine the efficacy of granulocyte transfusions and in what patient population they are useful.

MISSOURI, UNIVERSITY OF (N01-CM1-2323)

This is a development and resource contract to generate high resolution GLC, HPLC and other techniques and apply them to the analysis of markers in patient body fluid samples provided through NCI. Excellent methods have been perfected for polyamines, neutral protein-bound sugars, minor t-RNA nucleosides, and hydroxyproline. Normal ranges have been delineated and the validity of using casual urine samples and creatinine to replace 24-hour specimens has been proven. Large series of multiple markers in a number of kinds of cancer have been run and are currently being completed to evaluate these markers individually and in combination for usefulness in measuring progression or remission of disease as guides to chemotherapy. The developmental work is largely complete and it is expected that work on the current series of markers will conclude this year with phase out of the contract in September 1978.

MISSOURI, UNIVERSITY OF (N01-CM8-7157)

This contract will provide for a complete pathological, parasitical, and microbiological work-up of breeding stock primarily sent from the barrier room expansion colonies and the nude mouse production colonies.

MONSANTO RESEARCH CORPORATION (N01-CM6-7046)

This service preparative contract provides for the large-scale synthesis of compounds required for preclinical and clinical studies. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

MONSANTO RESEARCH CORPORATION (N01-CM7-7140)

This service preparative contract provides for the large-scale extraction of 40,000 lbs. of Maytenus plant. The extracts then have the active antineoplastic agent maytansine removed and purified. This contract will expire this year.

MONTEFIORE HOSPITAL (N01-CM6-7061)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM4-3703)

This contract provides 80 patients (previously untreated) with advanced ovarian cancer to study various drug combinations utilizing thiotepa, methotrexate, cytoxan, adriamycin and cis-platinum. Work to date has shown value of cis-platinum in combination with other agents and has verified

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM4-3703) (CONTINUED)

Phase II data of Wiltshaw. Also performing *in vitro* testing of cis-platinum and studying use of sonography as a staging and follow-up procedure. This contract terminated March 1978.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM5-3837)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies have been conducted on neocarzinostatin (NSC-157365), dichloromethotrexate (NSC-29630), maytansine (NSC-153858), m-AMSA (NSC-249992), PALA (NSC-224131), indicine-N-oxide (NSC-132319), and two drug combinations pyrazofurin plus azacytidine and 5-fluorouracil and thymidine, during the past year. This contract will terminate September 1978.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM5-3841)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM6-7096)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

MURPHY BREEDING LABORATORIES, INC. (N01-CM5-0579)

This contract furnishes approximately 312,000 six-week-old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

NATIONAL ACADEMY OF SCIENCES (N01-CM5-3850)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals; standards for nomenclature used to identify stocks and strains of laboratory animals; standards for methodology in maintaining axenic and gnotobiotic animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by *ad hoc* committees whose memberships represent commercial

NATIONAL ACADEMY OF SCIENCES (N01-CM5-3850) (CONTINUED)

animal production colonies, governmental and academic institutions, commercial laboratories, and non-profit research institutions.

NATIONAL ACADEMY OF SCIENCES (N01-CM5-7013)

This contract Task Order provides for conferences on diverse subjects that are intimately associated with the field of biomedical research. The agenda for specific meetings may be concerned with a common problem, or to develop the basis for a research program that is being considered by a segment of the scientific community. Conferences in the past have been held on research facilities, diseases of laboratory animals, professional training, the provision of animals for biological models for research, gnotobiotic technology, and requirements for standards in specific areas. Speakers are invited to present their thoughts and time is provided for open forum discussions. The proceedings of these conferences are published and made available to the scientific community.

NATIONAL NAVAL MEDICAL CENTER (Y01-CM5-0100)

The National Naval Medical Center has participated in programs furthering the understanding of the pathophysiology of breast cancer and in improving its treatment: (1) establishing a single multidisciplinary breast cancer clinic; (2) establishing joint therapeutic protocols in conjunction with those at the NCI; (3) involvement in joint laboratory studies relative to the detection and monitoring of breast carcinoma; and (4) publication and abstracts related to the contract. This contract also contributes to interdisciplinary efforts in testicular cancer and cooperates in the Eastern Cooperative Oncology Group protocols. NNMC has participated actively in the ECOG studies entering twenty-two patients.

NAVAL WEAPONS CENTER (Y01-CM5-0101)

This project will develop and evaluate prototype instruments for the quantitation of growth in granulocyte precursor cells. The monitoring technique closely follows that developed in missile guidance systems for remote detection of small targets against complex bright backgrounds. The National Cancer Institute and National Heart, Lung, and Blood Institute laboratories are collaborating in the design and evaluation of prototype instruments for ongoing Division of Cancer Treatment studies of leukopoietic mechanisms, the assessment of abnormalities in white cell proliferation, and the determination of marrow function associated with other diseases.

NEW ENGLAND MEDICAL CENTER (N01-CM6-7112)

This contract represents one component of a consortium of clinical centers studying the classification of non-Hodgkin's lymphoma. Because of the plethora of pathologic classifications in this area, the objectives of this collaborative effort are to collect and analyze retrospectively over 1000 cases of non-Hodgkin's lymphoma and correlate the clinical features (and lymphoid markers where available) with various pathologic classifications, seeking the major histologic determinants of clinical behavior. This contract will be phased out September 1978.

NEW YORK UNIVERSITY MEDICAL CENTER (N01-CM6-7059)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

NORTHEASTERN UNIVERSITY (N01-CM5-3741)

The development of CNS penetration antineoplastic agents is the primary goal of this contract. Compounds selected for synthesis consist of alkylating function attached to a known CNS active agent. Aminophenylisoquinolines and aporphines have been selected as the CNS active position. Structural modifications of the basic aporphine mustards will be determined by optimizing partition coefficients. This contract for the development of CNS antitumor agents will terminate this year.

NORTHERN CALIFORNIA CANCER PROGRAM (N01-CM8-7154)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The initial protocol is being written and will study the toxic effects as well as the therapeutic benefits of intensive preoperative chemotherapy and maintenance chemotherapy when added to a standard regimen of surgery and postoperative radiotherapy.

NORTHERN CALIFORNIA CANCER PROGRAM (N01-CM8-7154) (CONTINUED)

Controls will receive the standard surgery/radiotherapy regimen alone. Study sites will be limited to the anterior tongue, floor of the mouth and retromolar trigone/anterior tonsillar pillar areas in the oral cavity, and glottic larynx, supraglottic larynx and pyriform sinus in the laryngopharyngeal region. Only patients with Stage III and IV lesions will be studied, except for patients with Stage II pyriform sinus lesions.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM5-3828)

The purpose of this contract is to obtain information on the disposition and metabolism of new agents entering Phase I clinical trial. The Contractor has completed work on the metabolism and pharmacokinetics of cytembena in humans and has developed an HPLC assay for maytansine and a sensitive and selective electron-capture gas chromatographic assay for NSC-51143 1H-pyrazolo[2,3-a]-imidazole. In addition, pharmacokinetic studies in animals have been completed with NSC-51143 and five metabolites identified. The clinical pharmacokinetics and metabolism of this agent will be investigated during the next year.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM5-7015)

The immediate objective of this contract is to synthesize fraudulent sugars related to daunosamine. Daunosamine is an integral part of each of the two broad spectrum antitumor agents, namely, daunomycin and adriamycin. The Contractor designs and synthesizes configurational modifications of the sugar which is then coupled to daunomycinone, to form novel anthracyclines. Based on this concept the Contractor has successfully synthesized a number of daunomycin analogs for antitumor evaluation. The contract will terminate this year.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM6-7060)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM6-7060) (CONTINUED)

utilized as well as insight into the biologic characteristics of the disease entity being investigated.

OKLAHOMA, UNIVERSITY OF (N01-CM6-7108)

The major objective of this contract is the preparation, from animal materials of marine species, of extracts suitable for bioassay in the National Cancer Institute antitumor screen. The extracts are prepared in accordance with procedures acceptable to the National Cancer Institute. Extracts showing preliminary activity are reconfirmed and those materials showing confirmed activity are assigned for fractionation and isolation. This contract will expire this year.

ONTARIO CANCER INSTITUTE (N01-CM7-7152)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage I tumors are randomized (double blind trial) to intrapleural BCG + INH x 12 weeks versus placebo intrapleural BCG + placebo INH x 12 weeks. Resectable Stage II and III epidermoid carcinoma is randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

OREGON STATE UNIVERSITY (N01-CM2-3201)

The purpose of this contract is to acquire information on the disposition of drugs in animals so that such information is available prior to initiating clinical trials. Several nitrosoureas, some classical and some newer agents, are being studied from two points of view. First, the metabolic alterations and chemical degradation that these drugs undergo are being studied in detail with a view toward the effects of these changes on their biological properties. Second, the effects of these agents on the ability of the host hepatic microsomes to metabolize other drugs is under investigation. This contract will terminate July 1978.

PAPANICOLAOU CANCER RESEARCH INSTITUTE (N01-CM8-7230)

This contract will provide for a complete pathological, parasitical, and microbiological work-up of breeding stock primarily sent from the barrier room expansion colonies and the nude mouse production colonies.

PARKE, DAVIS & COMPANY (N01-CM7-7146)

This fermentation contract is designed primarily to obtain novel antitumor agents. This contract includes: (1) the preparation of fermentation beers from various microbes isolated from unique substrates from various parts of the world and fermented under a bevy of environmental and stress situations; (2) an *in vitro* tissue culture assay laboratory which assists in prescreening fermentation broths for cytotoxicity and is used to help assay chemical fractions, fermentation improvement samples and large pilot plant lots more quickly; (3) a small biotransformation program; (4) the isolation work required to obtain the active component from the confirmed active beers; (5) the production of large quantities of antineoplastic agents approved for clinical trials; and (6) the preparation of appropriate dosage forms of such agents.

PARKE, DAVIS & COMPANY (N01-CM8-7124)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 50% of the effort on this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

PENNSYLVANIA, UNIVERSITY OF (N01-CM3-3719)

This contract provided for designing and installing the Chemical Information System which supports the Developmental Therapeutics Program's compound accessioning activities by: (1) identifying which compounds are new to the Developmental Therapeutics Program, including the flagging of analogs and duplicates of compounds of interest; (2) producing computer-generated shipping documents and other communications to suppliers, screeners, in-house staff and contractors engaged in the acquisition, storage and distribution process; (3) maintaining inventory control; (4) searching both structural and nonstructural information using simplified, on-line inquiry procedures and providing structure output; and (5) maintaining management and operational control of the system. The system has been installed in the Chemical Abstracts Service (CAS) which is the Contractor operating the system. This contract terminated December 1977.

PENNSYLVANIA, UNIVERSITY OF (N01-CM5-3830)

The objective of this contract is to evaluate the acute and chronic effects of selected antitumor drugs on the energy performance status of isolated, perfused organs using both surface fluorescent and transmitted light absorbance methods developed by the Contractor. These models are to be applied to delineating the biochemical basis of either the therapeutic activity or limiting toxicity of selected drugs and their analogs. This contract terminated December 1977.

PFIZER, INC. (N01-CM5-3805)

The main purposes of this contract are to produce large quantities of mammalian type-C viruses in tissue culture and to purify and concentrate these viruses from the tissue culture fluids. In addition, the Contractor is to prepare certain viral components both to use in quality control of virus production and to analyze the human tissues for the presence of viral internal antigens by competition radioimmunoassays.

PHARM-ECO (N01-CM8-7200)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 70% of the effort of this contract is devoted to the preparation of large quantities of material, in the multi-kilogram range.

PHILIPS ROXANE LABORATORIES, INC. (N01-CM6-7053)

This service contract provides the Division of Cancer Treatment with facilities for development, formulation and production of oral dosage forms of investigational drugs. The dosage forms are manufactured in conformity to Good Manufacturing Practices. These dosage forms are packaged, labelled and shipped to the National Cancer Institute for subsequent distribution to clinical investigators.

PITTSBURGH, UNIVERSITY OF (N01-CB2-3876)

This study is designed to support the study of adjuvant treatment in breast cancer.

PITTSBURGH, UNIVERSITY OF (N01-CM7-7177)

This contract is designed to support randomized controlled studies using adjuvant therapy in treatment of colo-rectal cancer.

POLYSCIENCES, INC. (N01-CM7-7070)

This service preparative contract provides for the large scale extraction of various plants and isolation and purification of the active materials for preclinical development and clinical trials. Cost and yield data are obtained on these processes. In the case of those plant processes which will be used again, process development optimization studies are conducted.

POMONA COLLEGE (N01-CM6-7062)

This contract conducts quantitative structure-activity studies on congeneric series of antitumor agents. In addition, the contract provides experimental determination of necessary physicochemical parameters (log P, E_s, sigma) when these values are not available in the literature.

PUBLIC HEALTH SERVICE, DIVISION OF FEDERAL HEALTH PUBLIC SERVICES (TRANSFER OF FUNDS - Y01-CM4-0003) (W. HYATTSVILLE, MARYLAND)

This Transfer of Funds permits service support of the National Cancer Institute, Intramural Baltimore Cancer Research Program. This includes suitable laboratory facilities, support personnel and other administrative support. This contract will terminate September 1978.

PURDUE UNIVERSITY (N01-CM6-7091)

This contract provides for the fractionation of confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compound(s). Plant material used in this work is obtained, for the most part, through the United States Department of Agriculture.

RADIOBIOLOGICAL INSTITUTE, ORGANIZATION FOR HEALTH RESEARCH (N01-CM5-3763)

The objective of this contract is to conduct preclinical investigations of the combination modality of radiation and hypoxic radiosensitizers plus chemotherapy. Appropriate tumor test models are being explored and developed for this combination modality. With such models determination will be made of combinations and regimens of drug plus radiation that are optimal. The program is designed to provide an appropriate preclinical basis for the clinical application of radiation plus chemotherapy.

RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK (N01-CM5-3766)

This contract is to perform *in vivo* and *in vitro* basic investigation and development of new experimental models for studying the effects of anticancer agents on immune responses. Specifically, the effects on T and B cells, macrophages and the *Hh* systems will be assessed.

RESEARCH TRIANGLE INSTITUTE (N01-CM6-7089)

The primary purpose of this contract is to fractionate confirmed active plant extracts in an attempt to isolate and identify the active antineoplastic compound. In addition, active fermentation materials obtained from other contractors are isolated, purified and identified. The plants used for this work are primarily obtained from the United States Department of Agriculture. The Contractor has many plants which are or have been in some stage of

RESEARCH TRIANGLE INSTITUTE (N01-CM7-7089) (CONTINUED)

fractionation and several fermentation crude materials which are being purified. Several of these active agents have been isolated and are being evaluated more broadly. A KB cell culture facility is used to expedite fractionation work.

RESEARCH TRIANGLE INSTITUTE (N01-CM6-7121)

This service preparative contract provides for the synthesis of radioactive labeled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at the Institute or acquired from other sources, are analyzed for purity and identity by radioautography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff.

ROCHESTER, UNIVERSITY OF (N01-CM7-7159)

This contract supports the Radiation Oncology Coordination Subcommittee of the DCT Board of Scientific Counselors and its working groups to provide expert advice and recommendations to the DCT in the planning and development of radiation oncology research. This responsibility includes: a review and assessment of the state of the science in radiation oncology; development of a scientific research plan and recommendations for future research activity in radiation oncology to be presented to the Division of Cancer Treatment and its Board of Scientific Counselors; and to assist in fostering collaborative research among investigators within the program in the area of radiation oncology and therapy.

RUTGERS STATE UNIVERSITY (N01-CM6-7106)

This contract provides for the isolation of unique Black Yeasts and their polysaccharides in sufficient quantities for evaluation in the Drug Evaluation Branch *in vivo* solid tumor tests. The contract will expire this year

SAINT JUDE'S CHILDREN'S RESEARCH HOSPITAL (N01-CM7-7127)

The Contractor is synthesizing thiadiazole ribosides and pyrazofurin analogs as potential inhibitors of the salvage pathway enzymes. Effective agents of these types should permit selective destruction of cancerous tissue. This contract is in its final year and will be converted to the CREG mechanism.

SIDNEY FARBER CANCER INSTITUTE (N01-CM4-3781)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease.

SIDNEY FARBER CANCER INSTITUTE (N01-CM4-3799)

This contract was designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas. It is presently undergoing phase-out.

SIDNEY FARBER CANCER INSTITUTE (N01-CM5-3839)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies have been conducted on maytansine (NSC-153858), neocarzinostatin (NSC-157365), bruceantin (NSC-165563), PALA (NSC-224131) and 5-fluorouracil with thymidine during the past year. This contract will terminate September 1978.

SIDNEY FARBER CANCER INSTITUTE (N01-CM5-7035)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

SIDNEY FARBER CANCER INSTITUTE (N01-CM6-7037)

The Contractor conducts Phase II/III studies in patients with solid disseminated tumors. The tumors to be included are limited to: lung, breast, melanoma, soft tissue and bone sarcoma. A minimum of 400 patients a year, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy or surgery in protocols agreed upon by the Project Officer and Principal Investigator. Studies in nutritional support for patients undergoing systemic antineoplastic chemotherapy are also conducted under this contract. Major efforts in melanoma and in breast cancer have contributed to better regimens in the advanced stages of these tumors.

SIMONSEN LABORATORIES, INC. (N01-CM5-0578)

This contract furnishes approximately 312,000 six-week-old B6D2F1 (C57BL/6 female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

SIMONSEN LABORATORIES, INC. (N01-CM5-3847)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SIMONSEN LABORATORIES, INC. (N01-CM7-7166) (FORMERLY N01-CM5-3817)

The Contractor maintains a primary genetic center of inbred strains or rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production colonies from pedigreed expansion colonies.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (N01-CM5-3820)

The objective of this contract is to provide ^{125}I labelling of RNA from specific sources and the fingerprinting analysis of the nucleotide sequences of the ^{125}I-labelled RNA. These are needed for comparative sequence analysis of normal and human leukemic DNAs and for the comparative sequence analysis of laboratory type-C RNA viruses and candidate human viruses.

SMALL BUSINESS ADMINISTRATION (N01-CM4-3719)

The purpose of this contract is to provide supportive services in virology, immunology, and tissue culture. At the present time these functions include primarily the following: (1) detailed karyotypic analysis, including Giemsa banding, of a variety of monolayer and suspension cultured cells; (2) evaluation of tumorgenicity of various cultured cells by inoculation into nude mice; (3) testing the tumorigenic potential of selected primate retroviruses; (4) preparing and testing antiserum to selected primate retroviruses; (5) preparing small quantities of selected (frequently cloned) cells and retroviruses; and (6) testing various tissue cultured cell specimens for mycoplasma contamination.

SOUTH FLORIDA, UNIVERSITY OF (N01-CM8-7220)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The initial protocol is being written and will study the toxic effects as well as the therapeutic benefits of intensive preoperative chemotherapy and maintenance chemotherapy when added to a standard regimen of surgery and postoperative radiotherapy. Controls will receive the standard surgery/radiotherapy regimen alone. Study sites will be limited to the anterior tongue, floor of the mouth and retromolar trigone/anterior tonsillar pillar areas in the oral cavity, and glottic larynx, supraglottic larynx and pyriform sinus in the laryngopharyngeal region. Only patients with Stage III and IV lesions will be studied, except for patients with Stage II pyriform sinus lesions.

SOUTHERN ANIMAL FARMS (N01-CM5-0599)

This contract furnishes approximately 312,000 hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

SOUTHERN ANIMAL FARMS (N01-CM5-3845)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM4-3795)

This contract was designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas. It is presently undergoing phase-out.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM5-3842)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM6-7065)

The purpose of this contract is to develop a detailed pharmacokinetic profile of clinically useful antineoplastic agents. Thus far, a comprehensive model has been developed for adriamycin distribution in the rabbit, utilizing a multicompartmental analysis. This model, appropriately modified for physiologic parameters in man, has been applied to plasma pharmacokinetics in man and proved to be predictive of parent drug concentrations in plasma. Further collection and analysis of human data are planned, as well as initiation of similar studies of the human pharmacokinetics of the folate antagonists aminopterin and 3',5'-dichloromethotrexate.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM6-7084)

The Contractor is engaged in the synthesis of two types of potential antineoplastic compounds. These are the mono and dimeric forms of cyclopentenoisoquinoline for use as potential DNA intercalation agents, a novel "K cat" antagonist to act as a suicide inhibitor of dihydroorotate dehydrogenase, an important enzyme in the de novo path for pyrimidine synthesis. This contract effort is scheduled for conversion to the CREG program next year.

SOUTHERN RESEARCH INSTITUTE (N01-CM4-3756)

This project is designed to provide to DCT a maximally flexible single instrument for the rapid conduct of program directed developmental and applied studies pertinent to all pre-clinical, therapy related, tasks. Principal tasks are (1) to apply fundamental biological principles to the development of new and improved laboratory models for the discovery of more effective antitumor therapies including individual drugs, drug combinations, combined treatment modalities; (2) to evaluate, in detail, drugs in development to NCI sponsored clinical trial to determine their optimum conditions of usage; (3) to test new agents for activity against animal tumor and human tumor xenograft models which are not routinely used elsewhere in the DCT contract program; (4) to conduct routine in vivo and in vitro screening in response to program needs; and (5) to carry out specialized experiments to solve problems arising during large animal toxicology or early clinical trial of new drugs or to answer pre-clinical therapy related questions arising from FDA review of DCT sponsored IND applications.

SOUTHERN RESEARCH INSTITUTE (N01-CM4-3762)

The objective of this contract is to synthesize a variety of novel compounds as potential cancer agents based on biological and biochemical rationales. The three basic categories of compounds under investigation are nucleosides, antifolates, and chemically reactive types. Some of the enzyme systems for which inhibitors are being sought include deaminase, thymidine kinase, nucleoside diphosphate reductase and key folate enzymes. The synthetic program is well integrated with the screening, therapeutic, and biochemical efforts at the Institute. The contract will terminate this year.

SOUTHERN RESEARCH INSTITUTE (N01-CM4-3780)

The purpose of this contract is to obtain basic quantitative pharmacologic information in animals which will indicate the requirements of drug dosage and scheduling necessary to give maximum tumor cell kill while minimizing toxicity to the host's normal cells. This involves the development and application of highly sensitive analytical methodology to measure drugs and their metabolites in body fluids and tissues and the estimation of half-lives and cumulative Cxt values in animals and man. During the past year studies

SOUTHERN RESEARCH INSTITUTE (N01-CM4-3780) (CONTINUED)

have involved L-PAM, indicine-N-oxide, deoxycoformycin, phosphoramide mustard, piperazinedione, aminothiadiazole and bruceantin.

SOUTHERN RESEARCH INSTITUTE (N01-CM4-3784)

The major objective of this contract is to develop basic biochemical knowledge useful in the design of new drugs or in the more effective use of known agents. To accomplish this aim, projects are underway on (1) determining if new antitumor drugs have biochemical activities similar to those of clinically established drugs, (2) providing clear leads to biologically significant biochemical properties of new antitumor drugs, (3) evaluating the molecular mechanisms of action of new antitumor drugs, (4) developing systems to allow selection of structural analogs of clinically established antitumor drugs which have more desirable biochemical properties, and (5) developing biochemical rationales for the combined use of clinically established antitumor drugs. This contract terminated March 1978.

SOUTHERN RESEARCH INSTITUTE (N01-CM5-7000)

The principal objective of this contract is to compare the relative toxicity and therapeutic activities of cancer chemotherapeutic agents alone and in combination when different dosage regimens are used in rodent tumor systems and in non-human primates or dogs. These studies are intended to serve as a basis for the design of a combination drug regimen acceptable for clinical trial. The combination of L-phenylalanine mustard and 5-fluorouracil is currently under study.

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (N01-CM6-7075)

This contract provides *in vitro* testing of new natural products and of the fractionation products of crude materials which have shown activity in the *in vivo* tumor test systems of the Developmental Therapeutics Program. *In vitro* testing is carried out with the KB cell line and the P388 and L1210 lymphoid leukemia cell lines. Testing is conducted in accordance with the Developmental Therapeutics Program protocols and research and development studies are conducted at the request of the Project Officer. The current level of testing is approximately 5,000 KB test equivalents per year.

SPARTAN RESEARCH ANIMALS, INC. (N01-CM5-0582)

This procurement contract provides for the supply of 156,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for the Developmental Therapeutics Program compound evaluation studies. Breeding animals originate in genetic centers.

ST. LOUIS UNIVERSITY SCHOOL OF MEDICINE (N01-CM5-7020)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

STANFORD RESEARCH INSTITUTE (N01-CM3-3723)

Stanford Research Institute is one of the two facilities for the chemical analysis of experimental medicinal compounds and their clinical formulations for the Developmental Therapeutics Program. It determines the identity, purity, solubility, stability and other physiochemical properties of these materials. The techniques employed consist of elemental analysis, chromatography (paper, thin-layer, gas and liquid), spectrometry (mass, nuclear and photo), and other physical-chemical procedures appropriate to the situation. In the case of new structures the method for assay may have to be developed by the Contractor. The contract provides information as a guidance to the effective use, formulation and storage of the materials; provides quality assurance for the release of the materials for investigational use; and provides the necessary analytical data for filing with the Food and Drug Administration.

STANFORD RESEARCH INSTITUTE (N01-CM3-3742)

The research effort of this contract is directed toward the synthesis of new chemical structures. Nucleosides, antimetabolites, antibiotics, and alkaloids are the primary areas of chemical synthesis. No new initiatives in the anthracycline area were undertaken but the effort has been directed toward completing the studies previously undertaken. Other areas of synthesis include benzo[i]phenanthridine and ellipticine intercalators, mesoionic heterocycles, analogs of glyoxylic acid tosylhydrazone, analogs of jacaranone (an antitumor phytoquinoid), and analogs of the antitumor antibiotic meleimycin. This contract will expire during this fiscal year.

STANFORD RESEARCH INSTITUTE (N01-CM6-3776)

This service preparative contract provides for the synthesis of radiolabeled chemicals and drugs for use in preclinical pharmacologic and clinical studies. Many of the materials prepared are not available from commercial sources and are synthesized. All materials, whether prepared by the contract group or acquired from other sources, are analyzed for purity and identity by radioautography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff.

STANFORD RESEARCH INSTITUTE (N01-CM8-7207)

The primary goal of this contract is to develop a drug that is clinically useful as a radiosensitizer of radioresistant hypoxic tumor cells. The electron-affinic 2-nitroimidazoles with electron-withdrawing group substituents should provide potent radiosensitizers. The Contractor will synthesize compounds of interest to the radiobiologist and investigate some of the factors that affect the in vivo activity such as electron reduction potential, mammalian toxicity, hydrophobicity and drug distribution. Part of the contract will be carried out with Professor Martin Brown, Stanford Research Institute, who will determine the radiosensitivity in a number of hypoxy tumor-cells. Structure-activity will then provide guidance for the selection of compounds for synthesis.

STANFORD UNIVERSITY (N01-CP6-5825)

As part of a multi-institutional cooperative effort, the effectiveness of routine nutritional practice will be compared with that of total parenteral nutrition via central-line hyperalimentation. Fifteen pediatric patients with malignant tumors of the abdomen and/or pelvis requiring radiotherapy and intensive courses of multiple chemotherapeutic agents will be studied. Comparisons will be made with respect to: (a) tolerance to therapeutic schedule; (b) therapeutic response to combined chemotherapy and radiotherapy; (c) effectiveness of having the bowel "at rest" with respect to effects of therapy on gastrointestinal function; (d) nutritional status of the patient; and (e) complications during therapy. It is hypothesized that maintaining good nutritional status through total parenteral nutrition during combined radiotherapy to the abdomen and intensive chemotherapy, will result in fewer complications, decreased hospitalization time, and better therapeutic response. This hypothesis will be tested by a prospective randomized study comparing the patients maintained by total parenteral nutrition with those on routine dietary intake.

STANFORD UNIVERSITY, BOARD OF TRUSTEES (N01-CM6-7072)

This contract serves as a statistical support center to collect, analyze, and correlate the data submitted by the participating data collecting centers (New England Medical Center--N01-CM6-7112, University of Minnesota--N01-CM6-7113, Instituto Nazionale de la Cura dei Tumori--N01-CM6-7064, and Stanford University--N01-CM6-7072).

STANFORD UNIVERSITY, BOARD OF TRUSTEES (N01-CM6-7111)

This contract represents one component of a consortium of clinical centers studying the classification of non-Hodgkin's lymphoma. Because of the plethora of pathologic classifications in this area, the objectives of this collaborative effort are to collect and analyze retrospectively over 1,000 cases of non-Hodgkin's lymphoma and correlate the clinical features (and lymphoid markers where available) with various pathologic classifications, seeking the major histologic determinants of clinical behavior. This contract will be phased out September 1978.

STARKS ASSOCIATES, INC. (N01-CM5-3745)

This service contractor provides the Drug Synthesis & Chemistry Branch with support in the acquisition of chemicals and drugs for evaluation in the primary and secondary screens. The Contractor is required to: 1) provide liaison services to develop new sources of material and to encourage potential suppliers to participate in the program through voluntary contribution of samples; 2) collect samples; 3) provide support services in the processing of compounds; 4) aid in the management of computer generated reports, and routine correspondence with suppliers on subjects relating to compound acquisition; and 5) assist in the development and implementation of guidelines and methods of selecting materials for the screen.

STARKS ASSOCIATES, INC. (N01-CM5-3769)

This service contract is for the resynthesis of compounds not readily available from commercial sources or from the original supplier in the amounts needed. The compounds prepared are for the completion of definitive testing or for clinical or preclinical evaluation. About 50% of the effort of this contract is devoted to the preparation of large quantities of materials. Many of the preparations involve multistep sequences.

STARKS ASSOCIATES, INC. (N01-CM8-7203)

This service preparative contract is for the resynthesis of bulk chemicals and drugs required for completion of drug evaluation studies, with approximately 50% of the effort being devoted to the production of clinical materials. The materials assigned for resynthesis are not readily available in the quantities needed from the original supplier or on the open market. Preparations vary in quantity from gram to multikilogram scale.

STEHLIN FOUNDATION FOR CANCER RESEARCH (N01-CM6-7073)

This project provides the needed capability to study the growth characteristics, transplantability, genotypic and phenotypic constance, and response to selected antitumor drugs of human tumor xenografts grown in athymic ("nude") mice. Its major objective is to recommend to DCT human tumor xenograft lines to be used for screening. The Contractor conducts developmental studies leading to the formulation of precise protocols for screening against xenografts including parameters and criteria of drug activity. Athymic mice used in this project are bred by the Contractor under this contract. Data on all new human tumors developed as xenografts are submitted to DCT. These include patient's primary diagnosis, time of transplantation into "nudes" relative to diagnosis, patient treatment prior to presentation, further treatment and response, current number of passages in mice, change in xenograft growth pattern, percentage of "takes" in mice, extent of necrosis, maximum volume reached, metastases, and sensitivity to known chemotherapeutic agents.

TACONIC FARMS, INC. (N01-CM5-0597)

This contract furnishes approximately 260,000 B6D2F1 (C57BL/6 female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

TENNESSEE, UNIVERSITY OF (N01-CM5-7021)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

TEXAS, UNIVERSITY OF (N01-CM5-3767)

Sublines of murine neuroblastoma and astrocytoma cells (in culture) are developed to be resistant to antitumor agents of interest to the Division of Cancer Treatment. These sublines are examined with respect to mechanism

TEXAS, UNIVERSITY OF (N01-CM5-3767) (CONTINUED)

of resistance and pattern of cross-resistance to other antineoplastic agents. In addition, the efficacy of new anticancer agents is being examined in intact Ajax mice (syngenetic to the neuroblastoma clones) inoculated intraperitoneally or subcutaneously with neuroblastoma cells.

TEXAS, UNIVERSITY OF (N01-CM5-3832)

This contract is designed to investigate the role of protected environments (PE) and prophylactic antibiotics as adjuvants to the remission induction chemotherapy of non-Hodgkin's lymphoma, breast cancer in adults, and small cell cancer of the lung. Current clinical trial designs compare intensive chemotherapy plus PE with similar, conventionally administered, chemotherapy regimens.

TEXAS, UNIVERSITY OF (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM3-3710)

Provides 80 patients (previously untreated) with advanced ovarian cancer to study new agents. Work to date has shown hexamethylmelamine to be an active agent in ovarian cancer and possibly synergistic with alkylating agents.

TEXAS, UNIVERSITY OF (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM3-3737)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systematic and progressive understanding of the disease and promote its control. During the past contract year new drugs and new drug combinations have been explored in patients with distant metastases.
This contract expired April 1978.

TEXAS, UNIVERSITY OF (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM5-3773)

The purpose of this contract is to carry out studies on the pharmacology and physiological disposition of new antitumor agents under development by the National Cancer Institute. During the current year, studies have been completed on ftorafur, tricyclic nucleoside (NSC-154020), Baker's antifol, cisplatinum diammine dichloride, and adenine arabinoside when used in conjunction with the adenosine deaminase inhibitors 2'-deoxycoformycin and EHNA. Studies have also continued on the effects of bacterial immunoadjuvants on hepatic enzymes involved in antitumor drug metabolism. Studies under this contract are carried out in experimental animals and in cell culture lines, but are closely interrelated with Phase I clinical trials and clinical pharmacologic studies conducted at the M. D. Anderson Hospital and Tumor Institute and elsewhere.

TEXAS, UNIVERSITY OF (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM7-7149)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage I tumors are randomized (double blind trial) to intrapleural BCG + INH x 12 weeks versus placebo intrapleural BCG + placebo INH x 12 weeks. Resectable Stage II and III epidermoid carcinomas are randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinomas and large cell carcinomas are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

TEXAS, UNIVERSITY OF, MEDICAL BRANCH (N01-CM8-7221)

A member of the Head and Neck Contracts Program: A collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced resectable squamous carcinoma of the head and neck. The initial protocol is being written and will study the toxic effects as well as the therapeutic benefits of intensive preoperative chemotherapy and maintenance chemotherapy when added to a standard regimen of surgery and postoperative radiotherapy. Controls will receive the standard surgery/radiotherapy regimen alone. Study sites will be limited to the anterior tongue, floor of the mouth and retromolar trigone/anterior tonsillar pillar areas in the oral cavity, and glottic larynx, supraglottic larynx and pyriform sinus in the laryngopharyngeal region. Only patients with Stage III and IV lesions will be studied, except for patients with Stage II pyriform sinus lesions.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM4-3786)

The major emphasis of this project is to provide evaluation of the effects of new antitumor drugs on DNA biosynthesis or function, RNA synthesis, processing or function (ribosomal, messenger and transfer RNA) and incorporation into RNA or DNA. This contract terminated March 1978.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (N01-CM4-3801)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies have been conducted on neocarzinostatin (NSC-157365), deazauridine (NSC-126849), hycanthone (NSC-142982), dichloroallyl lawsone (NSC-126771), m-AMSA (NSC-249992), bruceantin (NSC-165563), imidazole pyrazolo (IMPY, NSC-51143), and PALA (NSC-224131), during the past year. This contract terminates September 1978.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM5-7042)

The Contractor conducts Phase II/III studies in patients with solid disseminated tumors. The tumors to be included are limited to lung, breast, melanoma, soft tissue and bone sarcoma, and gastrointestinal cancers. A minimum of 400 patients a year is studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy or surgery in protocols agreed upon by the Project Officer and Principal Investigator. Studies in nutritional support for patients undergoing systemic antineoplastic chemotherapy are also conducted under this contract. Major efforts in melanoma and in breast cancer have contributed to better regimens in the advanced stages of these tumors. Intravenous hyperalimentation has been shown to reduce myelotoxicity of chemotherapy.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (N01-CM6-7026)

Ovarian Cancer Study Group Pathology Reference Center: A special task contract to review surgical and postmortem specimens submitted by the Ovarian Cancer Study Group.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (N01-CM6-7116)

A collaborative group whose main thrust is to study various post-surgical adjuvants in early epithelial tumors of the ovary. A joint protocol for good risk Stage I (i.e., FIGO Stage IAi and IBi) compares observation to L-PAM post-operatively. A secondary study involves a histo-pathologic study of the incidence of unsuspected pelvic and/or paraortic node metastases in this patients population. A further joint protocol for other early disease (i.e., FIGO Stage IAii, IBii, IC, and II) separates patients according to presence or absence of macroscopic residual disease post-operatively. Patients with macroscopic residual disease are randomized to L-PAM versus L-PAM + pelvic radiotherapy. Patients without macroscopic residual disease are randomized to L-PAM versus intraperitoneal instillation of 15 mc of radioactive chromic phosphate.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM7-7153)

Lung Cancer Study Group Pathology Reference Center: A special task contract to review cytology, surgical biopsies, and autopsy material provided by the Lung Cancer Study Group.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CP6-5794)

As part of a multi-institutional cooperative effort, the effectiveness of routine nutritional practice will be compared with that of total parenteral nutrition via central-line hyperalimentation. Forty-four pediatric patients with metastatic bone disease requiring radiotherapy and intensive courses of multiple chemotherapeutic agents will be studied. Comparisons will be made with respect to: (a) tolerance to therapeutic schedule; (b) therapeutic response to combined chemotherapy and radiotherapy; (c) nutritional status of the patient; and (d) complications during therapy. It is hypothesized that maintaining good nutritional status through total parenteral nutrition during combined radiotherapy to the abdomen and intensive chemotherapy, will result in fewer complications, decreased hospitalization time, and better therapeutic response. This hypothesis will be tested by a prospective randomized study comparing the patients maintained by total parenteral nutrition with those on routine dietary intake.

UPJOHN COMPANY (N01-CM7-7100)

This contract has for its primary objective the research and development of potentially useful antineoplastic agents from fungi, bacteria and actinomycetes, fermentation beers, including isolation, purification, characterization, and production.

The Upjohn Company has played a leading role in the development and exploitation of cell culture methods, both for assays on fractionation samples and for primary screening. Leads developed from this screen are being tested in-house in the leukemia *in vivo* screen and actives are given top priority for chemical fractionation.

Major efforts in this contract are also devoted to biotransformation of known Upjohn antibiotics.

UTAH, UNIVERSITY OF (N01-CM5-3827)

The purpose of this project is to determine the pathologic effects of the combination of radiation and adriamycin therapy using the New Zealand white rabbit as a model. It has been well-documented that ionizing radiation can produce cardiac damage in both humans and in the New Zealand white rabbit. Similarly, adriamycin has been shown to produce cardiomyopathies in both humans and the New Zealand white rabbit. With the increased use of adriamycin in treating a variety of human malignancies, particularly when combined with radiation, the question is raised of the possibility of enhanced cardiac damage in radiation or drug dose ranges which have been previously considered to be safe. This contract will expire June 1978.

UTAH, UNIVERSITY OF (N01-CM7-7142)

The objective is the development of highly effective types of antitumor agents through the design and synthesis of purine nucleoside analogs. Under active investigation are the synthesis of: (1) analogs of tubercidin, sangivamycin, and toyocamycin; (2) derivatives of formycin; (3) pyrazolopyridine nucleosides; (4) imidazopyridine and triazolopyridine nucleosides; (5) imidazole and benzimidazole nucleosides; (6) pyrrolo pyrimidine nucleosides; and (7) unusual tri and tetracyclic nucleosides. The work is guided by biochemical-biological evaluation in collaboration with Dr. Robert E. Parks of Brown University.

VALUE ENGINEERING COMPANY (N01-CM5-3821)

This contract provides programming and technical support to the Investigational Drug Branch (IDB). The Contractor operates and maintains the Drug Distribution and Protocol Monitoring System (DDPMS). This automated system was developed to assist in the management of drug distribution authorization and investigational drug data gathering. As a consequence, incoming requests for clinical drug supplies are verified against an updated data base to insure accuracy prior to shipment. In addition, protocol data is recorded in the automated system permitting an organized retrieval, thus assisting the IDB in compliance with the Food and Drug Administration's requirements governing investigational drugs.

VALUE ENGINEERING COMPANY (N01-CM6-7086)

Data processing services are provided to the Developmental Therapeutics Program by this contract. The scope of work includes (1) reducing and disseminating information developed in the screening program of the Drug Evaluation Branch to both staff and the suppliers of the compounds being tested; (2) documenting all computer programs and contractor's procedures for data handling and running computer programs; (3) maintaining computer programs so that they are able to run in the Division of Computer Research and Technology environment; (4) modify the existing data system so that data from new antitumor systems can be handled; (5) refine the data collection methods; (6) providing instructions for screening laboratories and suppliers of materials relating to collection and dissemination of data; (7) providing output for statistical evaluation of test systems and evaluation of test system parameters; and (8) participation in scientific meetings.

VANDERBILT UNIVERSITY MEDICAL CENTER (N01-CM7-7122)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage I tumors are randomized (double blind trial) to intrapleural BCG + INH x 12 weeks versus placebo intrapleural BCG + placebo INH x 12 weeks. Resectable Stage II and III epidermoid carcinoma is randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

VERMONT, UNIVERSITY OF (N01-CM3-3716)

This contract provides for the extension of the Computerized Problem-Oriented Medical Information System (PROMIS) to the Division of Cancer Treatment area by developing and integrating structured input ("cancer displays") into this general medical information system. It is through the use of these explicit informational and directional cancer displays that the health care provider can couple learning with doing, while gathering information for individual cancer patients entered on problem-specific plans (cancer treatment research protocols).

VETERANS ADMINISTRATION (Y01-CM7-0107)

Under this Interagency Agreement is included the support for cooperative groups dealing with clinical investigations in various aspects of cancer treatment. One part of the effort consists of clinical trials done by the VA Surgical Adjuvant Group which evaluates chemotherapy, radiotherapy, or immunotherapy as adjuncts to treatment at the time of initial resection in lung, gastric, pancreatic, esophageal, rectal, colon, and head and neck carcinomas. The other part is that of the VA Lung Group which deals with the investigation of the multimodality treatment in advanced lung cancer. Ancillary studies include the identification of prognostic factors, the significance and evaluation of hormonal markers and histopathologic classification.

VETERANS ADMINISTRATION HOSPITAL (SOUTH CAROLINA) (Y01-CM6-0102)

The objective of this contract is to synthesize complexes of platinum which show high anticancer activity, good aqueous solubility and minimum renal toxicity. Current effort is focused on a number of exploratory approaches to synthesize selective metal complexes including oxidation state modifications, and incorporation of novel ligands of the complex. In animal tests, a number of these compounds have shown activities superior to the parent dichlorodiammine platinum (II).

WALTER REED ARMY MEDICAL CENTER (Y01-CM8-0108)

This Interagency Agreement is in support of Walter Reed Army Medical Center (WRAMC) participation in Cancer and Leukemia Group B as a full group member. WRAMC has participated actively in all the protocols of the CALGB entering 85 patients in studies during 1977. The Principal Investigator and staff have attended all meetings and are developing multimodality functions in medical oncology, pediatrics, pathology and radiotherapy. WRAMC staff generated six new protocols during 1977 and 183 new patients were entered on WRAMC protocols. The Principal Investigator has been involved in planning of the Head and Neck Study Program and the Testicular Study Program. He is also a medical oncologist in the Gynecology Oncology Group.

WARF INSTITUTE, INC. (N01-CM4-3707)

This is a service contract to prepare extracts of plant materials for screening by the screening laboratories. The great majority of plant extracts screened by NCI originate with this contract. The majority of the Contractor's plants are obtained through a Transfer of Funds Agreement between the National Cancer Institute and the United States Department of Agriculture. Special studies of methodology of plant extraction are conducted in order to maximize the yield of confirmed active plants.

WARF INSTITUTE, INC. (N01-CM5-3770)

This is a service contract for *in vivo* screening of potential anticancer agents. Currently, testing is being conducted at a level of approximately 50,000 L1210 equivalent tests per year. Agents tested include new crude plant extracts, fractionations of previously confirmed natural products, new synthetic materials, and follow-up testing of active materials. Testing is conducted in mice in accordance with National Cancer Institute protocols. The P388 lymphocytic leukemia is employed as the model for the initial testing of all materials. Additional models now in use under this contract include the following transplantable mouse tumors: the B16 melanocarcinoma, the $CD8F_1$ mammary, colon 26, colon 38, ependymoblastoma, L1210 lymphoid leukemia, Lewis lung carcinoma, Madison 109 lung carcinoma, and the 16C variant of the C_3H mammary. These tumors are transplanted for testing into various sites including subcutaneous, intravenous, intraperitoneal, intracerebral, and intramuscular sites. Schedule dependency studies are conducted for materials of potential clinical interest. Methodology and/or research and development, as well as special research projects, are scheduled as the need arises and are directed by the Project Officer.

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE (N01-CM4-3731)

The cytocidal effects of single drugs on normal hematopoietic stem cells and malignant cells (L1210, AKR, B-16 melanoma, and Lewis lung) are quantitated by using a spleen colony or lung colony assay. Drug pairs are then evaluated

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE (N01-CM4-3731)

for their effects on tumor and host cell survival. From these data principles for drug-pair sequence and dose intervals may be disclosed. This contract terminated March 1978.

WAYNE STATE UNIVERSITY (N01-CM6-7105)

The Contractor conducts Phase II/III studies in patients with solid disseminated tumors. The tumors to be included are limited to lung, breast, melanoma, soft tissue and bone sarcoma. A minimum of 400 patients a year are studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy or surgery in protocols agreed upon by the Project Officer and Principal Investigator. Studies in nutritional support for patients undergoing systemic antineoplastic chemotherapy are also conducted under this contract. Major efforts in melanoma and in breast cancer have contributed to better regimens in the advanced stages of these tumors.

WISCONSIN, UNIVERSITY OF (N01-CM6-7076)

This contract provides rapid cell culture cytotoxicity assays as an aid in isolation and purification of potential antitumor agents of microbiologic origin. Standardized assays are performed in accordance with procedures described by Developmental Therapeutics Program protocols employing KB, L1210, and P388 cell lines. Occasional methodology studies may be carried out in consultation with the Project Officer.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM4-3797)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM5-3824)

The objective of this contract has been to determine whether differences which exist in plasma membranes between normal cells and those transformed by oncogenic viruses and chemical carcinogens can be exploited with chemotherapeutic agents. An exploratory program of synthesis of membrane carbohydrate analogs and the evaluation of their effects on growth inhibition and surface properties of the membranes of neoplastic cells was initiated. The analogs have the potential to be incorporated into glycoproteins and glycolipids in the membranes and thus modify the immunogenicity and/or oncogenicity of the tumor cells. This contract terminated December 1977.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM5-3843)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM5-7029)

The purpose of this contract is to develop new antitumor agents which interfere with either the synthesis or the function of nucleic acids. To accomplish this objective, a series of diacridines has been designed and synthesized based on the rationale that they would act as bifunctional intercalators, bind more strongly to DNA than acridine derivatives and thereby inhibit DNA related reactions. The diacridines are evaluated for their ability to bind to DNA, and to inhibit cell growth *in vitro*, as well as for their effect on other cellular functions. These properties are compared with the *in vivo* antitumor effectiveness of the diacridines in order to determine whether correlations exist. This contract terminated April 1978.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM6-7068)

The purpose of this contract is to screen new antifols, obtained through NCI, for their inhibitory activity against human dihydrofolate reductase. Further studies of potent inhibitors are conducted to determine whether they have enhanced selectivity for the neoplastic cell based upon differential enzyme inhibition or transport characteristics. When indicated, pharmacological studies, directed toward the establishment of unique properties which would justify a clinical trial of a selected drug, are carried out in experimental animals. This contract will terminate June 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06401-06 OD

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Data Processing Systems for Scientific and Management Information

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Gordon	Systems Analyst	OD DCT NCI
OTHER:	L. Greene	Computer Specialist	OD DCT NCI

COOPERATING UNITS (if any)
Data Management Branch, Division of Computer Research and Technology, National Cancer Program, Management Information System

LAB/BRANCH

SECTION
Office of the Director, Division of Cancer Treatment

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Cancer Treatment Program is stratified for purposes of monitoring, analyzing and planning the allocation of resources. A stratification form is used by DCT staff to collect, aggregate, and tabulate monthly budgetary information. This information is then evaluated periodically to determine the adequacy of funding for each R&D line item.

The raw data for this DCT budget management system is obtained by the automation of contract data input via RCB, NCI; direct operations data input via DFM and the NCP-MIS; and cooperative group grants information via DCRRC, NCI. The stratification forms have been designed to capture Disease- and Modality- oriented budget information, which is then applied to the financial data. Differentiation between "Research" and "Support" is also indicated.

With the assistance of DMB, DCRT; a mailing label system has been developed. This system has been used primarily for the mailing of the "DCT NEWSLETTER" and will be utilized in the future for the consolidation of the diverse mailing lists now in use at DCT.

PHS-6040
(Rev. 10-76)

Project No. Z01-CM-06401-06-OD

Project Description

Objectives: 1. To establish a complete DCT budget management system by the automation of contract data input via RCB, NCI: direct operations data input via DFM and the NCP-MIS; and cooperative group grants information via DCRRC, NCI.

2. Revision of the DCT Stratification to provide comprehensive Disease-Oriented and Modality-Oriented budget information.

3. Establishment of a microfiche information system for archival and distribution purposes.

4. Development of a mailing label system.

Methods Employed: 1. Through the use of DMB, DCRT program generation software, a single file containing all DCT contracts (commitments, Schedules A&B), direct operations data, and grants data is updated monthly at the computer terminal. This update is via the DMB, DCRT UPDATE and EDIT system; the data is entered into the WYLBUR Reformat Generator (WYLGEN) for conversion to the appropriate transaction format. This update is designed for use by non-programmers. All entries in the file are classified with respect to the DCT Stratification. Differentiation between "Research" and "Support" is also indicated.

2. Stratification forms have been designed to capture Disease - and Modality-Oriented budget information. Programs have been written by DMB, DCRT for the maintenance of the DCT data file by use of this information. Contractural information procurement continues as before, with the TRW-generated linkage to the RCB, NCI contract management system for monthly updates. Cooperative Group grants information is now obtained from DCRRC, NCI via WYLBUR and from DRG via the IMPAC system.

3. Monthly reports are now distributed and stored on microfiche.

4. With the cooperation of the Special Assistant to the Director, DCT, NCI: a consolidated DCT mailing label file is maintained and updated when necessary.

5. A Financial Data Report Subsystem (FDRS) has been developed by TRW Systems Group and the NCP - MIS. The FDRS generates monthly financial reports used by DCT administrative officers and their staffs to monitor actual versus planned expenditures. These reports provide the information necessary to support management decisions relative to budget and spending admustments.

Major Findings: 1. Programs have been written by DMB, DCRT to generate reports by various combinations of the DCT Stratification elements applied to contract, direct operations, and grants data as well as by DCT organization. Several reports are available for classification of the DCT budget by the DCT Stratification. Emphasis is placed on Disease-and Modality-oriented summary reports.

2. The FDRS now produces reports that were previously done manually by the Administrative Officers with the use of DFM reports. The following information is processed by the FDRS: (a) Obligation and commitment data obtained from the Allotment Ledger Master (ALM) File of the NIH Central Accounting System (CAS), (b) Apportionment data supplied by the DCT Administrative Officers and their staffs, (c) Cost center codes, project codes, CAN's and titles pertinent to the division.

3. Regeneration of the DCT data file has allowed for the incorporation of many new variables, which pertain to the Cooperative Group grants that have been transferred from DCRRC. Accordingly, many new budget reports may soon be generated upon request.

4. The availability of contract and DCRT account data has enabled this office to formulate the annual ADP plan requested by the OD, NCI. Statistics pertaining to the Data Processing expenditures of DCT are now available via the "new" DCT Stratification as applied to the DCT budget.

Significance to the Program of the Institute: The computer system that has been developed with the assistance of DMB, DCRT is utilized to monitor and assess budget information related to segments of the DCT program and to more specific project areas both in the intramural areas and in the contract program. The reports generated by this system lead to a detailed description of the DCT research program.

Proposed Course: Possible modifications of the stratification form to allow for the receipt of treatment data from other divisions of NCI is contemplated. These modifications will, in turn, require another large scale revision of the information system with the subsequent generation of new reports by DMB, DCRT.

The FDRS is based upon the use of the report generation packages IRS (Inquiry and Reporting System) and TPL (Table Producing Language). These packages were utilized to minimize development and future revision costs. Automation of direct operations data input to the DCT Stratification files may be feasible via alteration of the IRS and TPL programs of the FDRS. This requires further investigation.

Many duplicate records must be eliminated from the mailing label file. At the same time, the identifying "flags" that are appended to each record must be updated. This is a major task, but it must be done in order to utilize the mailing label system for the consolidation of the diverse mailing lists now in use at DCT. At this time, the mailing label system is used primarily for the distribution of the "DCT NEWSLETTER".

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07025-04 OD

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biomedical Engineering for Intramural Research, DCT

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: W. Penland, Biomedical Engineering Project Officer, OD, DCT, NCI

OTHER: DCT NCI V. DeVita, B. Lewis, R. Atkinson, T. Strike (OD); A. Deisseroth, P. Pizzo (PO); R. Magin, S. Shackney (LCHPH); R. Young, R. Ozols (MB); P. Bunn, M. Cohen (VAMO); S. Schimpff, P. Weirnik (BCRC); P. Sarin, R. Gallagher (LTCB); V. Bono (ID); L. Kirkland (RO); S. Rosenberg, P. Sugarbaker (SURG); others.
Arthur D. Little, Inc. A. Wechsler (Program/projects); C. Kensler and staff (Mgt. Review); J. Lyddy, D. Lindsay, E. Cook, R. Liss, A. Neilson, others (Projects).
Los Alamos Scientific Laboratories (ERDA) P. Mullaney, G. Salzman (PI's).
Oak Ridge National Laboratories (ERDA) J. Breillatt, C. Remenyik (PI's).
U.S. Naval Weapons Center M. Benton, D. Sticht (PI's).
Artek Systems Corporation C. Leonhardt (PI).

COOPERATING UNITS (if any)
RTI/NASA Technology Utilization, R. Scearce; LTD, NHLI, P. Carmesi; Indianapolis Center for Advanced Research, F. Fry.

LAB/BRANCH
Office of the Director, Division of Cancer Treatment

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 7.0	PROFESSIONAL: 6.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Collaborative biomedical engineering resources have focused upon the characterization and implementation of useful engineering projects for a diversity of intramural needs in: tumor cell kinetics, hyperthermia studies, specialized clinical instrumentation and products, and blood cell procurement and preservation.

Project Description

General Objective/Scope

1. To provide broadly based as well as specialized biomedical engineering (BME) resources to the research program of the Division of Cancer Treatment, NCI.

2. To identify and characterize problems amenable to BME solutions and to conduct BME research and development efforts responsive to the diverse research programs, projects and tasks of individual investigators of the Division of Cancer Treatment and collaborating groups.

3. To develop and refine approaches for the effective management of broad BME resources through the contract mechanism.

Clinical Instrumentation and Projects

An improved colonoscope using automated instead of manual advancement could potentially form the basis of a labor-effective procedure for diagnosis of colonic pathology, especially for those identified through widely used screening techniques for colorectal cancer (Hemocult Test). During this period, four prototype rubber cuff devices, attached to a colonoscope and capable of being alternately inflated and deflated and advanced in a peristaltic fashion, have been developed and partially tested (Lyddy, ADL; Sugarbaker, Penland, DCT). Trials in dogs, using manual and automatic advancement have been promising. Accordingly, efforts are presently focused on a minimum risk protocol for the simplest device to be examined in five patients undergoing abdominal surgery. Movement and control of the device will be observed directly in the presence and absence of bowel peristalsis which will be controlled by drugs. Confirmation of its working principle and better visualization of the large bowel is expected.

The quantitation of growth in colonies of marrow granulocyte procursors for studies of abnormalities in white cell proliferation (Bull, Atkinson, Penland, DCT) has been supported for four years by prototype devices developed by the U.S. Naval Weapons Center in collaboration with NHLI. Techniques for the automatic measurement of spatial frequency components of bone marrow colonies in a gelatin field are being exploited. A much improved second prototype was developed by USNWC and evaluated by DCT during this period. Except for improvements affecting the uniformity and quality of data output, which are now being made, this device appears to meet the original objective of accelerating day-to-day experimental bone marrow colony assay and of improving the statistical significance of such work.

The application of focused ultrasound techniques to transiently modify the blood brain barrier for enhanced drug transmission has been partially examined this period (F. Fry, Indianapolis Center for Advanced Research; Wodinsky, ADL; Penland, Strike, Walker, DCT). Early experimental work by

Dr. Fry has shown that a high intensity focused ultrasonic beam can be transmitted through an excised adult skull and used to produce thermal flaws in a simulant for live brain tissue. Also, Trypan blue, with a molecular weight comparable to several chemotherapeutic drugs, has been transmitted through the region of irradiation in the rat brain. Exploratory efforts are now being considered for transmission of BCNU in a rat or mouse glioblastoma model using advanced instrumentation.

The ADL project to improve the flavor characteristics of oral antibiotics (Neilsen, ADL; Schimpff, BCRC) achieved modifications in Vancomycin, Garamycin, and Tobramycin that resulted in blander more palatable flavors and improved patient acceptance in early trials. The Vancomycin work was most noteable. Prior to further improvements with artificial flavors, this modified agent is preferentially being given to patients at the present time. Modifications to Nystatin have not been successful to date.

Engineering assistance was given in all phases of procurement for the new laminar flow facility at Baltimore Cancer Research Facility during this period (Schimpff, BCRC; Penland, DCT).

Assistance with modified commercial equipment for replacement of the NCI biological isolation garment has continued this period with the introduction of "tubed foods" and additional noise control measures (Pizzo, Penland, DCT; Hollender, Natick R&D Command).

To broaden DCT interest in the control of pain in cancer patients, a presentation of an extensive experience in applying and evaluating electrical neuromodulation devices was accomplished this period (Penland, Rosenberg, DCT; Burton, Sister Kenny Institute).

Blood Cell Procurement and Presentation

The initial goal of the ORNL Project to achieve a high yield rotor for granulocyte collection via continuous flow centrifugation was mostly accomplished last period during intensive clinical trials (Deisseroth, Ungerleider, NCI; Breillatt, Remenyik, ORNL). Two new rotors have been produced this period and production drawings made available to commercial interests. Government patent coverage was accomplished and the excellence of the rotor design was recognized by Industrial Research (International R&D Journal) as one of the 100 most significant new technical products of the year.

Granulocyte cryopreservation and microwave thawing studies at Emory University and Georgia Tech were evaluated this period (Deisseroth, Applebaum, Penland, DCT) for possible sponsorship by DCT. Similarly, prospective studies by ORNL (Mazur) of membrane related properties believed important to granulocyte cryoprotectants were also evaluated and subsequently awarded support for a 2 year period.

Hyperthermia Projects

Engineering support for the Phase I evaluation of hyperthermia and radiation therapy protocols by the RTOG Program was continued this period. The ADL staff has selected, furnished and partially evaluated microwave and thermometry equipment packages for six RTOG organizations.

Provision was made for Bureau of Radiological Health specialists to contribute directly to RTOG Subcommittee interests in the technical monitoring, calibration and design of certain thermometers and microwave applicators, and in the diagnosis and optimization of microwave energy deposition in clinical procedures via thermography.

Cell Kinetic Instrumentation and Studies

Commercial development was completed this period for a TV based cell scanning system to complement flow microfluorimetry (FMF) studies of tumor cell classification and related interests (Shackney, Bunn, others, NCI). This system semi-automatically obtains the integrated optical density of the cell nucleus through silouetting techniques and counts autoradiographic grains within the cell nucleus or cytocytoplasmic areas on a cell-by-cell basis. Data display can take multiple forms. This system represents the first economical semi-automatic image processing unit for accelerating cell kinetic studies of the NCI and collaborative groups. The first two prototypes will be evaluated by DCT (Shackney, Bunn, Penland, DCT) during 1978. Commercial versions are expected to follow.

Flow microfluorometry (FMF) instrumentation from the Los Alamos Laboratories during this period covered: (a) continued improvement in advanced data processing software, (b) on-site technical support for correcting FMF malfunctions in flow chambers, cell sorting hardware and electronics lines of DCT interest and (c) publication and reporting of technical progress and results. A renewal agreement which continues similar activities was approved this period.

Interest in techniques for high speed autoradiograph development for cell kinetic studies exists in several DCT areas (Young, Ozols, Shackney, Bunn). Accordingly, detailed investigations were made this period of novel processing approaches involving: O_{17} labeled thymidine with registration by nuclear track emunlsion techniques (Carpenter, NBS; Slatkin, Brookhaven); tritium decay and registration by electrical breakdown of gas in spark chambers (Malmon); and radiographic toners for image inhancement (Shapiro).

Significance

This biomedical engineering activity relates to a number of exploratory research areas in the Division of Cancer Treatment that can potentially benefit from bioengineering approaches. Such activity enhances the scope and data base of the biological and medical scientist to achieve specific research goals. It thereby increases the probability that new engineering methodologies and instrumentation will contribute as in the past, to short and long range goals of the Division of Cancer Treatment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09053-03 OD

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Physical Science Collaborative Research

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

E. R. Atkinson, Physical Science Project Officer, NCI/DCT/OD
Y. W. Kim, Cancer Science Fellow, NCI/DCT/LTCB
P. Mahendroo, Cancer Science Fellow, NCI/DCT/LTCB
J.M.C. Bull, Senior Staff Member, NCI/DCT/MO
D. A. Van Echo, Senior Staff Member, NCI/DCT/BCRC
R. Johnson, Chief, Radiation Therapy, Roswell Park Memorial Institute/RTOG

COOPERATING UNITS (if any) Nat'l Aeronautics & Space Adm., Food & Drug Adm., Nat'l Bureau of Standards, Dept. of Defense, Presidential Office of Telecommunication Policy, Energy Research & Development Adm., Environmental Protection Agency, Occupational Safety & Health Administration.

LAB/BRANCH
Physical Science Project Office

SECTION
N/A

INSTITUTE AND LOCATION
Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Physical science collaboration is being conducted in areas of: automated tissue culture colony counting; millimeter wave spectroscopy of tumor cells; whole body hyperthermia patient treatment; whole body hyperthermia animal studies; toxicity of chemotherapeutic agents at elevated temperatures; non-thermal effects of microwaves; precision measurement of size of biological objects; mass spectroscopy of biopolymers and unnatural isotopes; nuclear magnetic resonance of membrane specific proteins; human thermoregulation; receptor site tertiary structure by electron spin resonance; dielectric properties of normal and tumor tissues; pion production at low energies in the laboratory frame; tissue sensitizers for ionizing radiation; microwave imaging techniques; ultrasonic temperature measurement; liquid crystal effects in biological materials; optical and viscometric techniques for temperature measurement; actin and myosin mechanics.

PHS-6040
(Rev. 10-76)

REPORT OF THE ASSISTANT DIRECTOR FOR INTERNATIONAL TREATMENT RESEARCH

OFFICE OF THE DIRECTOR

The international activities of the Division of Cancer Treatment are continuing to develop rapidly. They are being coordinated by the Office of the Assistant Director for International Treatment Research which was established in 1976 within the Office of the Director, Division of Cancer Treatment. In the office are A. Goldin, Assistant Director for International Treatment Research, O. Yoder, NCI Collaborative Office Representative, Brussels; J. Stoychkov, Eleanor Roosevelt Visiting Scientist; Makato Morimoto, visiting fellow and B. Nolte Secretary and M. Gelboin Part-time Clerk-Typist. Dr. Ira Kline Expert Consultant was located in this office until January 1978. Dr. T. Spiro of the Department of Clinical Pathology of the Clinical Center, NCI, has received an appointment of one year to the Institut Jules Bordet as a Visiting Fellow.

The international office coordinates the interests of the various program areas of the Division of Cancer Treatment in their relationships to international treatment research. This involves the development and continuing conduct of collaborative preclinical and clinical programs, monitoring of international developments in treatment, exchange of scientific personnel and information, procurement and exchange of synthetic agents and natural products for screening and development, and the identification of new compounds of potential clinical interest. Working closely with the Associate Director's Office for International Affairs, NCI, the program continues to maintain close contact with international organizations including the International Union Against Cancer (UICC), the World Health Organization (WHO), the European Organization for Research and Treatment of Cancer (EORTC), the International Agency for Research on Cancer (IARC) and the Pan American Health Organization (PAHO).

The Cancer Chemotherapy Research Collaborative Office at the Institut Jules Bordet (Chemotherapy liaison office), in Brussels has been providing a valuable multifaceted service to the Division of Cancer Treatment International Program. This Liaison Office headed by Dr. Omar Yoder, represents a reference center for researchers and cancer specialists in the United States and Europe, providing pertinent information for cancer research programs on both continents. The office is a primary instrument in interaction with European pharmaceutical and chemical industries in Europe and in the collection and evaluation of potentially useful antitumor agents.

The Collaborative Office is now well known by the scientific community in cancer research in Europe and is accepted as an intermediary link between the two scientific communities, Western Europe and North America. In this respect, it is fulfilling an irreplaceable function in bringing together leading cancer investigators of the two continents for exchange of ideas and latest developments. The office is fulfilling the important function of an observation center where European scientific news and events in the Cancer field are gathered and communicated to interested parties in the U.S. Conversely, this has now become the office to which Europeans turn to obtain first hand information on scientific advances and developmental approaches in the United States in cancer research. The "liaison" function of the office has been most useful in the areas of experimental and clinical pharmacology, clinical trials and in the organization of joint American-European workshops and symposia.

During the last year, approximately 3500 new materials selected from 15 countries, from more than 100 different European sources, were processed through the chemotherapy office for antitumor screening. There were 100 compounds with confirmed activity under the NCI guidelines. A significant number of new active compounds has undergone secondary investigation and two of these, a 9-hydroxy ellipticine derivative (NSC 264137) and a zinc complex of 2-carboxaldehyde thiosemicarbazone (NSC 294721) are currently undergoing Phase I clinical trial in Europe. A new cis-pt-derivative, NSC-269608, is planned for Phase I clinical trials in the near future. This compound could have the advantage over other compounds in the program in that it has reduced renal toxicity at the effective therapeutic dose level in animals. Eighteen publications have resulted from the joint effort with the Institut Jules Bordet during the past year. About 50 clinical trials are ongoing in Europe and the EORTC Data Center has over 5000 cases in its active file. The protocol review committee, which includes NCI representation, evaluates the ongoing protocols on a continuous basis, which facilitates a rapid exchange of information with the NCI programs. Collaborative programs continue with the Mario Negri Institute, the National Tumor Institute and the School of Medicine in Milan, the University of Perugia, the Institute of Cancer Research in London, the University of Leeds, the Laboratories of the Medical Research Council in Dublin, the TNO Institut in Rijswijk, the Institute of Cellular Pathology in Louvain, the Hospital St. Pierre in Brussels, and with various other institutions in Western and Eastern Europe. During this year new contacts have been established with laboratories and hospitals in Spain and Greece. Of significant importance is the expansion of the collaborative program with the Institute of Cancer Research (Chester Beatty) in London with Dr. Harrap's group.

During the last year a foreign exchange nurse program was implemented. Two nurses have spent 6 months at the NCI, one from the Royal Marsden Hospital in England and one from the Utrecht Hospital in the Netherlands. A clinical center NCI Nurse plans to visit the Royal Marsden Hospital during this year. The objectives of this program are: exchange of techniques and information in nursing research and collaborative participation in ongoing research programs. This program is designed to elevate the level of nursing research participation as well as care in oncologic therapy.

Progress was made during the year on the various bilateral programs in the administration of which close contact is maintained with the Office of International Affairs.

FRANCE

The French-American Agreement for clinical trials and treatment research, involving the Division of Cancer Treatment, NCI and the Institut National de la Sante et de la Recherche Medicale (INSERM) continues to make very good progress. The "Clinical Trials and Therapeutic Research" delegations within the program held a joint meeting at the INSERM headquarters in Paris on December 7-8, 1977, at which all six projects were reviewed. These include:

(1) Pharmacology of 5-FU and MTX. This program has been making excellent progress. Drs. Mihich and Chabner have been working closely with Dr. Meyer at his Cancer Center in Marseilles in the training of a team of pharmacologists. A workshop is planned for July 1978 in Marseilles on the pharmacology of 5-fluorouracil. Also, a training fellowship has been arranged for Dr. Luccione for investigation of biochemical aspects of 5-fluorouracil on target tissues, at Roswell Park Memorial Institute. Clinical trials in this program include comparison of high dose methotrexate and 5-fluorouracil by push versus infusion. It is planned to expand this program to other centers in Ville Juif.

(2) Nitrosoureas. This area involves primarily the Ville Juif group and Georgetown in extension of structure-activity studies. The nitrosourea derivatives RFCNU (French), RPCNU (French) and chlorozotocin (USA) are being investigated both in the laboratory and the clinic. RFCNU and RPCNU have yielded responses against various types of GI cancer and chlorozotocin has been active in melanoma and lymphoma in preliminary studies. Phase II studies are proceeding with chlorozotocin in the USA and with all three agents in France.

(3) Plasmapheresis. In this collaboration between Drs. Israel and Edelstein (Bobigny) and Macdonald (Georgetown) a Phase I study is being completed with entry of six patients from the United States and 7 from France.

(4) GI Cancer: close collaboration has been developed in this area between Georgetown and the groups headed by Drs. Boiron, Lagarde and Israel. The Ville Juif group may join these studies which would increase the number of patients substantially. Protocols include: a) adjuvant chemotherapy of gastric cancer with 5-fluorouracil, adriamycin and mitomycin C (FAM); b) treatment of advanced gastric cancer with ftorafur, adriamycin and mitomycin (FAM) (II) (furam); c) combination chemotherapy of pancreatic cancer with 5-fluorouracil, adriamycin and mitomycin C (FAM). The addition of chlorozotocin to the FAM regimen was considered. Also under consideration is the exploration of combinations with the French nitrosoureas. Since there is a relatively high incidence of gastric cancer in France, adjuvant trials in this disease involving new chemotherapy are proposed.

(5) Platinum compounds: A Phase II study involving cis-platinum diammine dichloride (DDP) is continuing at Ville Juif and in six additional cancer centers in close relation to the Cancer Therapy Evaluation Program. Activity in testicular cancer has been confirmed and encouraging results are being obtained in the treatment of cervical cancer. This has led to the initiation of combination chemotherapy with adriamycin, vincristine, bleomycin, cytembena, and cis-platinum for the treatment of advanced epidermoid cancers. In addition to Dr. Hyatt, Drs. Israel and Boiron have become involved in this project. Dr. Israel has reported on an effective combination employing DDP in testes tumors and preliminary activity in bladder and prostate cancer.

DDP plus cyclophosphamide was effective in preliminary studies in ovarian cancer. Investigations will also be conducted at Ville Juif with the diammine cyclohexyl malonate platinum derivative which is of potential interest in the treatment of hematologic malignancies.

(6) Anthracyclines: Interesting data are accumulating on the anthracycline derivative AD-32 and on diethoxy acetoxy-14-daunorubicin (DEA-DNR). The proponents of these drugs are Drs. Frei and Jacquillat respectively. DEA-DNR has demonstrated activity in the treatment of cutaneous metastasis and hematologic malignancies. Among solid tumors, sarcomas and thyroid cancer appeared responsive. Collaboration is projected in this area to include Japan and Italy, and a workshop on new anthracyclines involving these countries is planned for September.

In addition to the six ongoing projects, 8 additional projects are in various stages of development as follows:

1. Phase I and II immunotherapy and immunomanipulation involving experimental preclinical immune adjuvant screening, and immunity in screening of cytostatics.
2. Osteosarcoma
3. New Phase II studies
4. Hyperalimentation
5. Resistant breast carcinoma
6. Cyclic nucleotides
7. Liposomes
8. Hyperthermia, radiosensitizers, and hemi-body radiation.

Two of these, immunomanipulation and osteosarcoma will be funded by INSERM for 1978.

The travel requirements, both past and present have been fairly extensive, reflecting the strong interest in this program.

A meeting of various members of the USA-French Agreement will be held on the occasion of the EORTC plenary program at the end of June, and a meeting of the overall program coordinators has been scheduled for November 1978 in Bethesda.

Emphasis is being placed in this program on:

1. Long range research collaboration;
2. Organization of small workshops under the auspices of the cooperation, for intensive study of specific subjects;
3. Joint publication of results from cooperative studies;
4. Exchange of information involving therapeutic protocols;
5. Development of screening systems for assessing immunotherapeutic and immunosuppressive action of drugs, and of immunotherapeutic approaches.

USSR

The publication earlier of the USA-USSR Monograph "Methods of Development of New Anticancer Drugs" provides a significant collaborative accomplishment.

Definitive progress has been made in this program in preclinical areas. To date there has been an exchange of 165 drugs for preclinical evaluation; 61 American drugs have been given to the scientists in the Soviet Union and the American scientists have in turn received 104 Soviet drugs.

The Soviet drug ftorafur, a slow release form of 5-fluorouracil continues to be under clinical investigation in the United States. It would appear to have equivalent activity to 5-FU but with lesser myelosuppression.

The anthracycline derivative carminomycin is being investigated clinically in the Soviet Union, generally in combination with other drugs. It is not clear at this point whether carminomycin does have diminished cardiotoxicity as compared with adriamycin or daunomycin. Preclinically, however, it has been demonstrated to be active on oral administration.

Additional Soviet drugs of current program interest include prospidine, and dioxadet.

The collaborative monograph entitled "US-USSR Preclinical Antitumor Chemotherapy Test Data and Clinical Correlations" is in the final stages of preparation, and it is projected that it will be completed in 1978. Included in this monograph are the results of antitumor studies of 30 USA and 30 USSR drugs in a spectrum of US and USSR tumor systems. The primary objective of the monograph involves the analysis of the collective data. The following problems have been addressed: a) comparison of the data obtained employing the same methodological approaches with both common and different model tumor systems; b) conduct of an analysis of the experimental data in relation to clinical activity in order to extablish the possibility for prospective prediction of antitumor activity of new drugs in the clinic both for antitumor activity in general and for activity against specific categories of tumor. For this purpose, biological, biochemical and mathematical approaches are being employed, taking into account not only the data collected in each country but also the common data; c) employment of the analysis for the improvement of screening systems in each country. Included in the monograph in addition to the presentation of the test data analysis, is a listing of the drugs employed, their structures and physico-chemical characteristics and a description of the tumor systems.

It is considered that the results of this collaborative investigation may provide significant input into the screening methodology and to future investigations and approaches for obtaining new effective drugs for the clinic. It is of particular pertinence to the new prospective screening program recently instituted in the Division of Cancer Treatment, NCI, involving murine tumor models and human tumor xenografts growing in athymic mice.

Currently, the preclinical test data are being updated both for the compounds already incorporated in the monograph and the new ones that have been introduced into the exchange program.

In the preclinical areas joint planning of additional drug exchange and collaborative conduct of experimentation on specific problems in chemotherapy has been pursued on an ongoing basis.

In the clinic, in retrospect, the initial attempts to establish highly sophisticated joint protocols in the treatment of specific categories of human neoplasia probably occurred too early, prior to the establishment of a firm basis for collaborative effort in the clinical program. On the other hand, the cooperative clinical investigations and clinical information exchange are progressing satisfactorilly and are providing a sound basis for the potential development of pertinent joint protocols. The cooperative clinical investigations and clinical information exchange have included three working groups, namely breast cancer, lung cancer and ovarian cancer and this has de facto been extended to encompass the totality of clinical chemotherapy including essentially the entire range of neoplastic disease categories.

Clinical studies with USA anticancer drugs in the USSR may be summarized as follows:

a) Melanoma: actinomycin D plus vincristine plus methylnitrosourea as effective as actinomycin D plus vincristine plus CCNU plus DTIC, and more effective than actinomycin D plus vincristine plus CCNU;

b) Lung cancer: cyclophosphamide and methyl CCNU show equivalent activity to cyclophosphamide and CCNU;

c) Breast cancer: hexamethylmelamine and ftorafur individually active;

d) Stomach cancer: CCNU plus ftorafur and ftorafur alone comparable or superior to CCNU alone, 5-FU alone and CCNU plus 5-FU.

e) GI cancer: streptozotocin plus ftorafur and streptozotocin plus 5-FU have given encouraging preliminary clinical results;

f) Ovarian cancer: the high degree of activity of the NCI combination HEXA-CAF has been confirmed and equivalent activity was observed when ftorafur was substituted for 5-FU.

DTIC has received preliminary approval from the Soviet Council on Pharmacology and this undoubtedly will result in widespread use in clinical practice.

In the program there has been continuing exchange of clinical protocols, and joint planning.

The Fourth Joint American-Soviet Meeting on the Problem Area "Malignant Neoplasia" was held in Moscow September 25 through October 1, 1977, with Drs. A. Upton and Prof. N. N. Blokhin, as Coordinators of the cooperation. At the plenary session there was a review of the present status of the cooperative program in cancer chemotherapy and of the clinical aspects of the program. Sectional meetings were also held for the drafting of the appendices for the memoranda on specific "problem areas" including the problem area "cancer chemotherapy." There was a review of the plans for cooperation for the next year by the Joint USA and USSR representatives and various memoranda including the one on "Problem Area Cancer Chemotherapy" were signed.

A visit was made to the USSR by A. Goldin, I. Kline, and I. Miller January 14-21, 1978 including travel to Moscow and to Riga, Latvia to work with Drs. A. Syrkin, Z. P. Sofina and staff and the mathematicians A. M. Petrovsky and Vapnick on the finalization of the joint USA-USSR Monograph entitled "USA-USSR Preclinical Antitumor Chemotherapy Test Data and Clinical Correlations." This trip helped to complete the monograph and all that remains pertains to editorial procedures. In Riga the visit was to the Institute of Organic Synthesis where there was review of the scientific program, with particular attention to current studies with ftorafur and analogs. Dr. Rosenblitt of the Institute of Organic Synthesis described a computer method which is in the process of progressive development for the prediction of structure-biological activity in the synthesis of analogs of known drugs and new chemical structures. This is a novel and interesting approach and may provide an additional basis for future collaboration.

There was a tentative arrangement that a number of compounds with biological activity at the Institute be submitted for testing for antitumor efficacy under the USA-USSR agreement and it is hoped that this will be done soon.

The drugs, funkeosid, betalbosid and actinoxanthin were brought back from Moscow.

JAPAN

The USA-Japan Cooperative Cancer Research Program involving the Japanese Society for the Promotion of Science and the National Cancer Institute has continued to contribute in exchange of information and in the setting up of collaborative protocols in the project areas: gastric cancer, nitrosourea derivatives, bleomycin and its derivatives and anthracycline antibiotics. The collaborative gastro-intestinal study group program also continues to make good progress.

A symposium on antitumor antibiotics and the Third Annual Program Review of the USA-Japan Agreement on Cancer Research were held on May 12-13, 1977 in San Francisco. At the symposium, antitumor antibiotics of major interest that were discussed included mitomycin C, bleomycin and analogs, alone and in combination therapy, macromomycin, anthracycline derivatives and neocarzinostatin. At the business meeting, in addition to general review of progress to date there was review of the status of work on nitrosourea and bleomycins as well as a working session on the US-Japan Joint Protocol for Advanced Gastric Cancer.

The Eighth International Symposium of the Princess Takamatsu Cancer Research Fund was held November 15-17, 1977, on "Advances in Cancer Chemotherapy."

Following this on November 18 a meeting of the US-Japan Cooperative Cancer Research Program was held on new antitumor agents, with particular focus on new water-soluble nitrosoureas and aclacinomycin A. Areas involved in discussion both at the Symposium and the US-Japan Cooperative Cancer Research Program included: The National Cancer Institute New Screening Program, bleomycin and derivatives, adriamycin and related anthracyclines, approaches to improvement of anticancer activity with mitomycin C, pharmacology of nitrosourea derivatives, cyclocytidine and its mode of action, clinical and pharmacological aspects of fluorinated pyrimidine chemotherapy, clinical oncologic studies with platinum compounds, clinical-pharmacologic investigations with formyl leurosine, studies on the mode of action of neocarzinostatin, chemotherapy of testicular tumors, combination chemotherapy of lung cancer, treatment of ovarian cancer and treatment of childhood solid tumors.

Adriamycin has been shown to be active in gastric cancer by the Japanese. In Sendai they were employing the combination of adriamycin + ACNU + 5FU. At the Aichi Center in Nagoya they have a new regimen called A-MFC involving adriamycin and MFC (Mitomycin C, 5-Fluorouracil, Ara C) plus coenzyme Q10. A new adjuvant study at the National Cancer Center involves surgery with a three arm study involving PSK; ftorafur; ftorafur + PSK. All of these are preceeded by mitomycin C one day after surgery. At the Tokyo Metropolitan Hospital they are studying adriamycin, 5FU and mitomycin C.

Aclacinomycin has been entered into phase II study and a new fluorinated pyrimidine, FD-1 is in phase I study. Bestatin is of considerable interest as an immune stimulant.

A US-Japan joint symposium on Phase I and II trials in cancer chemotherapy was held June 5-6, 1978 in Tokyo. The major areas included (a) methodology, and Phase I and II trials; (b) the clinical evaluation of analogs, (c) clinical evaluation strategy in specific disease types, (d) new drugs, (e) four specific sessions on drug classes of interest, (f) the US-Japan joint gastric cancer protocol. Some drugs of interest that were discussed included 1-hexyl carbamoyl 5-FU, FD-1, ftorafur derivatives that releases 5FU slowly, bestatin, ACNU, bleomycin derivatives including pepleomycin, Yoshi 864, carbazilquinone, mitomycin C, neocarzinostatin, azotomycin, a series of nitrosourea derivatives, N^4-behenoyl ara C, cyclocytidine, and a wide variety of natural products, as well as plant products and other materials.

In the advanced gastric protocol the common arm is adriamycin plus 5-fluorouracil. The Japanese arm is adriamycin, ftorafur and mitomycin C. The American arm is BCNU, adriamycin and ftorafur.

The two Japanese contracts were quite active during the past year and through them the NCI program has access to the resources of the Pharmaceutical houses, chemical industry, universities and research institutes in Japan. The collaborative office for Cancer Therapy Research at the Japanese Foundation for Cancer Research in Tokyo, under the direction of Sakurai and Tsukagoshi continues to serve as the primary liaison in Japan. It has contributed in drug collection, and screening and evaluation, clinical investigations with new drugs, monitoring of new drugs in clinical trial and information and personnel exchange. The data obtained in the screening program are sent to DCT for entry into the computer program.

The contract with the Microbial Chemistry Research Foundation, Tokyo, under the direction of Umezawa is involved in the study of antitumor agents from microbial sources. It is largely because of this contract, with J. Douros as project officer that potential antitumor agents from natural sources become available to the DCT program at an early date.

LATIN AMERICA

The USA-PAHO-Latin American Program with Dr. Muggia as the principal DCT clinical representative has been making considerable progress.

A meeting was held on December 15, 1977 at PAHO headquarters in Washington, including the NCI and PAHO officials and the principal investigators in the United States. During this meeting guidelines were set for the coordination of submission of protocols for approval by the NCI and for the shipment of drugs to Latin American investigators. In addition the activities performed by each of the collaborating centers was summarized.

A second meeting was held April 5-6, jointly with the South American, USA and PAHO participants in Washington in conjunction with the ASCO and AACR meetings, for further review of protocols, and progress made, and also provided an opportunity to set up definitive methods for reporting of results and for arrangement of drug shipments.

An updated list of Latin-American Centers participating in the program and the corresponding U.S.A. counterparts is as follows:

COLLABORATIVE CANCER TREATMENT RESEARCH PROGRAM

LATIN AMERICAN CENTERS PARTICIPATING WITH THEIR CORRESPONDING U.S. COUNTERPARTS

LATIN AMERICA	Collaborating with	UNITED STATES
ARGENTINA		for ARGENTINA
a) Hospital Militar Central (HMC) Buenos Aires Dr. Roberto Estevez		a) Georgetown University Division of Medical Oncology Vincent T. Lombardi Cancer Research Center Washington, D.C. Dr. Philip Schein Dr. Paul Woolley
b) Academia Nacional de Medicina (ANM) Buenos Aires Dr. Santiago Pavlovsky		b) National Cancer Institute Bethesda, Maryland Dr. Franco Muggia Dr. John Ziegler
c) Instituto Angel H. Roffo (IAHR) Buenos Aires Dr. Americo Olivari		

COLLABORATIVE CANCER TREATMENT RESEARCH PROGRAM (cont'd.)

LATIN AMERICAN CENTERS PARTICIPATING WITH THEIR CORRESPONDING U.S. COUNTERPARTS

Collaborating with

LATIN AMERICA	UNITED STATES
BRASIL	for BRASIL
a) Instituto Nacional de Cancer (INC) Rio de Janeiro Dr. Cesar Camara Lima Santos	a) M.D. Anderson Hospital and Tumor Institute Houston, Texas Dr. Gerald Bodey
b) Fundacao A.C. Camargo (FACC) Sao Paulo Dr. Fernando Gentil	b) Memorial Sloan Kettering Cancer Center New York, New York Dr. Joseph Burchenal
CHILE	for CHILE
a) Universidad Catolica de Chile (UCC) Santiago Dr. Juan Arraztoa	a) University of Wisconsin Department of Human Oncology Madison Dr. Guillermo Ramirez
COLOMBIA	for COLOMBIA
a) Instituto Nacional de Cancer (INC) Bogota Dr. Julio Ospina	a) Roswell Park Memorial Institute Buffalo, New York Dr. Edward Henderson
PERU	for PERU
a) Instituto Nacional de Enfermedades Neoplasicas (INEN) Lima Dr. Eduardo Caceres	a) University of Miami School of Medicine Comprehensive Cancer Center for the State of Florida Miami Dr. Francisco Tejada

The current PAHO protocols (16) and approval dates are as follows:

CURRENT PAHO PROTOCOLS AND APPROVAL DATES

ARGENTINA

(10/6/77)	A randomized trial of two different chemotherapeutic regimens, BACOP or C-MOPP, in treatment of advanced diffuse histiocytic lymphoma (Pavlovsky, Woolley)

CURRENT PAHO PROTOCOLS (Cont'd)

ARGENTINA (Cont'd)

(10/6/77)	Phase II trial of chlorozotocin in leukemias, lymphomas, and other hematologic malignancies (Pavlovsky, Woolley)
(1/5/78)	Adjuvant chemotherapy and immunotherapy for squamous cell carcinoma of the esophagus (Olivari, Woolley)
(1/5/78)	Randomized trial of radiation plus 5-Fluorouracil versus streptozotocin, mitomycin C and 5-Fluorouracil in locally unresectable pancreatic cancer (Estevez, Woolley)
To be revised: (Reviewed 11/29/77)	Adjuvant chemotherapy and immunotherapy for squamous cell carcinoma of the oral cavity, oropharynx and larynx, Stages II, III, and IV

BRAZIL

(10/20/77)	Chemotherapy of disseminated breast cancer comparing FAC with FAC plus tamoxifen (Araujo, Bodey)
(1/5/78)	Cis-dichloro-diammineplatinum-11 (DDP) or cytoxan combination for advanced head and neck cancer (Araujo, Bodey)
(12/19/78)	Cis-platinum and bleomycin in epidermoid carcinoma, with special reference to head and neck, cervix, and penis (Gentil, Burchenal)
(In review-5/25/78)	Combination chemotherapy for advanced head and neck cancer (Cabral, Bodey)

CHILE

(1/16/78)	Protocol to study the effectiveness of chemotherapy and hormonal manipulations in women with disseminated breast carcinoma (Arroztoas, Ramirez)

COLOMBIA

(10/6/77)	Combination chemotherapy in disseminated or recurrent malignant melanoma (Ospina, Henderson)
(9/14/77)	A study of adjuvant chemotherapy with adriamycin versus adriamycin plus cis-platinum in osteogenic sarcoma (Ospina, Henderson)

PERU

(1974-closed)	Radical amputation and prophylactic radiation to lungs and chemotherapy with adriamycin in patients with resectable osteogenic sarcoma (Caceres, Tejada)

CURRENT PAHO PROTOCOLS (Cont'd)

PERU (Cont'd)

(1974-to be closed)	Simple mastectomy, combination chemotherapy and control in patients treated with radiotherapy for Stage III adenocarcinoma of the breast (Caceres, Tejada)
(12/16/77)	Primary combined treatment for patients with resectable osteogenic sarcoma (Caceres, Tejada)
(12/29/77)	Chemotherapy for local and metastatic Stage IV uterine cervix carcinoma and for recurrent Stages II and III uterine cervix carcinoma (single agent versus combined chemotherapy) (Caceres, Tejada)
(3/9/78)	Phase II study of procarbazine in metastatic measurable adenocarcinoma of the breast (Caceres, Tejada)
(To be revised-3/9/78)	Chemotherapy and radiotherapy versus chemotherapy and surgery and postoperative radiotherapy with or without immunotherapy in management of Stage III breast cancer (Caceres, Tejada)

Guidelines are being formulated for selective exchange of specialized nursing personnel.

A number of visits of the US investigators were conducted during the year to their Latin American counterparts for the planning of collaborative projects and for evaluation of facilities.

On August 1-17 Drs. Franco Muggia and A. Goldin visited Cancer Research Institutes in Brazil, Argentina, Uruguay and Peru, in relation to the collaborative cancer program. Institutes visited include:

1. Instituto Nacionale de Cancer, Rio de Janeiro, Brazil.
2. Instituto Central Hospital A. C. Camargo, Fundacion Antonio Prudente in Sao Paulo, Brazil.
3. The Department of Biochemistry at the University of Sao Paulo, Brazil.
4. Hospitals Santa Monica and Santa Casa in Belo Horizonte, Brazil.
5. Servicio de Oncologia de Hospital Militar Central in Argentina.
6. Servico de Hematologia de la Academia Nacionale de Medicina, Argentina.
7. Instituto Angel Roffo of the University of Buenos Aires, Argentina.
8. Municipal Hospital for Oncology in Buenos Aires, Argentina.

9. Municipal Hospital Guillermo Rawson, Buenos, Aires, Argentina.

10. The Department of Oncology of the University Hospital in Montevideo, Uruguay.

11. Seguro Social de Peru, Hospital Central No. 2 in Lima, Peru.

12. Instituto Nacionale de Enfermedades Neoplasicas (INEN) in Lima, Peru.

The above visits provided an opportunity for exchange of information pertaining to collaborative program development: In Argentina and Uruguay we were accompanied by Dr. Paul Woolley of the Division of Medical Oncology of Georgetown University.

During this trip Drs. Muggia, Woolley and Goldin participated in the Third Argentina Oncology Congress August 9-12, 1977.

CUBA

Participation in the Fifth Cuban Congress on Oncology and the First for the countries neighboring the Caribbean, from March 16th to the 27th, provided an opportunity to become familiar with preclinical and clinical cancer investigation in Cuba and to form a framework for possible future cooperation. The Congress was organized by the National Institute of Oncology and Radiobiology in Havana, Cuba, of which Prof. Zoilo Marinello Vidaurre is the director. He is also president of the Committee of Science and Technology for Cuba. The program in Cuba involved pre-congress courses followed by the Congress proper, with participation from various countries of the Americas and Socialist countries. A. Goldin visited the laboratories and clinical facilities of the Institute of Oncology and Radiobiology which has a number of young, dynamic and highly motivated investigators. Preclinical investigations are being conducted in various biochemical areas and in in vitro and in vivo investigations of chemotherapeutic activity of antitumor agents. There is a well-organized plant and marine collection program in a search for new antitumor agents, which is of interest to our program and which may serve as a basis for collaboration. They have a number of active plant materials that they are investigating, several of which may be new structures. In the Institute a number of new laboratories are under construction as well as a new animal facility which is nearing completion. They are interested and are planning to incorporate human tumors growing in athymic mice in their program. The clinical facility and program in radiotherapy and pediatric oncology are impressive. A new facility for experimental chemotherapy is close to completion, and it would appear important for their development of this program to have input from the USA.

An informal meeting was held with Dr. Zoilo Marinello and Dr. Daniel Joly who is the head of PAHO (WHO) in Cuba, concerning the possibilities for further cooperation and collaboration. Since our program is already involved in an NCI-PAHO-Latin American Program in Cancer Therapy and since Cuba is a member of PAHO it was considered that this avenue could be explored as a means of furthering scientific interrelations in cancer therapy. The Cubans are Specifically interested in exchanges of personnel and in collaboration in both preclinical and clinical areas. Perhaps the greatest immediate potential interest would lie in some form of collaboration in the natural products area where the Cubans have a well-developed program. They are interested in sending us some of their active materials for confirmation of antitumor activity, with a possibility of our helping with formulation and toxicological characterization with a view to introduction of interesting materials into Phase I trials. It would appear that interchange of personnel, particularly for further training, both in preclinical and clinical antitumor investigations, toxicology and pharmacology would be highly useful. Some aspects of collaboration in Phase I trials could be encouraged as the Cuban program develops.

UNITED KINGDOM

Cooperative preclinical and clinical research associations have been strengthened during the year between the USA and the United Kingdom, in view of the growing interest and collaboration of investigators in these two countries. A meeting was held at Leeds Castle, England, October 6-8, 1977, with a threefold objective: (a) to identify areas of mutual interest between the USA and UK in the fields of drug development and testing; (b) to recommend specific USA-UK collaborative projects of high priority; and (c) to establish improved lines of communication between cancer researchers in the USA and Britain related specifically to information and personnel exchanges.

It was agreed that USA-UK collaboration could begin in the following areas of mutual interest:

1) drug development and screening - The rational design of new drugs and analogs with increased selectivity requires experimental models with greater relevance to human malignancy. Such models would be of particular importance in identifying determinants of drug sensitivity and the mechanisms of resistance. For this purpose, there is considerable interest in the human tumor xenograft in the nude mouse and the need for validation of this model as a predictor for clinical responses. The UK has made an investigative committment to answer this question. One experiment agreed upon is to study single drugs in the clinic (phase 2 design) and in a corresponding nude mouse xenograft model using a single responsive human tumor (eg. head and neck cancer, ovarian cancer). It was agreed that attempts should be made to employ xenograft models in the study of drug sensitivity, resistance and other biochemical variables in the laboratory;

2) preclinical toxicology - there are similarities and differences in the toxicological evaluation of new drugs and analogs in the UK and USA, and it is considered of mutual interest that the toxicological resources in both countries be shared in the preclinical evaluation of drugs of mutual interest (eg. deoxycoformycin, penta-methylmelamine) for the efficient and expeditious movement of new drugs to the clinic;

3) clinical phase 1-2 testing. As in the USA the UK workers have established a close working relationship between the disciplines of clinical oncology and biochemical pharmacology and have the potential for developing and testing new drugs in a single environment. It is therefore considered mutually beneficial to exchange approved drugs of common interest for clinical testing and simultaneous pharmacologic study;

4) drug rescue strategies and scheduling - the various drug rescue schemes for averting bone marrow and gastro-intestinal toxicity are under consideration including: bone marrow autografting, high dose methotrexate and leucovorin and/or thymidine/hypoxanthine rescue. The UK clinicians may join a current USA study of high-dose/low-dose methotrexate with leucovorin rescue in head and neck cancer.

The UK workers have a highly interesting observation that low "primary" doses of cyclophosphamide or cytosine arabinoside enhanced the recovery from toxicity resulting from high doses of the same or different drugs administered several days later. Continued preclinical and clinical exploration of this phenomenon should be encouraged;

5) collaborative pharmacological and clinical testing of new drugs under development will be encouraged. This involves drugs such as deoxycoformycin, pentamethylmelamine and PALA.

The Leeds castle workshop provided an excellent format for discussion and agreement of collaborative investigations, and plans are currently under consideration for a second meeting to be held in the USA. Thus, collaborative ties already exist and are now being strengthened and broadened in scope. A supplement is being provided to contract no. N01-CM-43736 with the Institute of Cancer Research (with Harrap) of which John Venditti is the project officer, and which is now being joined by Dr. John Ziegler as the co-project officer.

SPAIN

In Spain there is considerable interest in the development of collaborative investigation with the United States. An opportunity was provided to make direct contact in Spain by an invitation to attend an international seminar on clinical oncology in Torremolinos (Malaga) on February 7-10, 1978, organized by the Spanish Society of Oncologic Chemotherapy. Through attendance at the International Seminar and visits in Madrid to the Ciudad Sanitaria de la Seguridad Social Primero de Octubre which is part of the Ministry of Trabajo, Instituto Nationale de Prevision and to the National Center for Medical Research at the Puerta de Hierro and a visit to the Foundation Jimenez Diaz, a start has been made in the direction of working up a cooperative relationship with Spain. As a follow-up, Dr. Yoder visited Dr. Mario Gosalvez at the National Center for Medical Research at the Puerta de Hierro and has made contact with Dr. Hernan CortesFunes at the Social Security Hospital and Dr. J. Vicente at the Foundation Jimenez Diaz as well as representatives of other institutions in Spain. It is planned that the effort here will be related directly with the USA in terms of possible cooperative effort and also via activity in EORTC.

EAST BERLIN

An opportunity was also afforded to make contact with cancer investigation in East Berlin, German Democratic Republic, through participation in an International Workshop on "Surgical Adjuvant Antineoplastic Chemotherapy in Solid Tumors", February 13-14, 1978. In East Berlin at the Central Institute of Cancer Research of the Academy of Sciences of the German Democractic

Republic at Berlin-Buch an opportunity was afforded to view a well-balanced clinical and research organization in which laboratory activities are closely related to the clinical divisions. The current organization of the Institute is relatively new since the director, Dr. Tanneberger took over the leadership only a few years ago. It may be pointed out that he is a vigorous and imaginative investigator with excellent associations in Cancer Therapy both in Eastern and Western countries, and the overall program can be expected to develop rather rapidly within the next few years.

HUNGARY

We continue a good association with the Cancer Institute in Budapest, Hungary. With Dr. S. Schepartz, I attended the First International Symposium on Cytostatic Galactitol derivatives in Budapest on September 24, 1977. This meeting provided an updated review of the current status of laboratory activities with dibromodulcitol, dianhydrogalactitol, dibromomannitol and other hexitol derivatives.

POLAND

In the US-Poland binational cooperative program there has been exchange of information and intervisits of scientific personnel. Dr. C. Radzikowsky will spend 3 months in the U.S. starting in July with Drs. Goldin and Chirigos at NCI and with Dr. E. Mihich at Roswell Park Memorial Institute. During this visit it is planned to work out a joint program in specific areas of immunochemotherapy.

FEDERAL REPUBLIC OF GERMANY

In connection with the projected collaborative program in Drug Development and Testing for Cancer Therapy with the Federal Republic of Germany we have now heard from Dr. H. Brieskorn of the Ministry of Research and Technology to the effect that the names suggested as scientific coordinators are:

Prof. Dr. N. Brock, Bielefeld
Prof. Dr. C. G. Scmidt, Essen
Prof. Dr. D. Schmäl, Heidelberg

The coordinators will contact Dr. A. Goldin as soon as the draft of the program is available. We are awaiting further word on this.

EGYPT

The bilateral program between the US and the Republic of Egypt has made progress during the year under Dr. John Ziegler's leadership. A paper was presented at the ASCO meetings in Washington in March describing initial collaborative Phase II trials in Bilharzial bladder cancer. This is an ongoing program with the objective of discussing active drugs and improved modalities. The ASCO abstract (1978) summarizes the progress made and is included as follows:

PHASE II CHEMOTHERAPY TRIALS IN CANCER OF THE BILHARZIAL BLADDER

Nazli Gad-El-Mawla, Reda Hamsa, Eric Chevlen, and John Ziegler
Cairo Cancer Institute, Cairo, Egypt, and
National Cancer Institute, Bethesda, Maryland 20014

Cancer of the bilharzial bladder is predominately of the squamous cell type. While the tumor tends to remain localized in the pelvis, patients present frequently with advanced, inoperable tumors which require chemotherapy. In an attempt to discover active drugs, a series of phase II studies was undertaken in sequential patients with inoperable bladder cancer in Egypt using agents which are effective in squamous carcinomas of other sites. The results of the first four trials are tabulated below:

Drug	Dose/Route	Schedule	No. Evaluable Pts.	Results CR	PR	NR
HMM	200 mg/m^2/d p.o.	daily >30 d.	26	0	10	16
VM-26	100 mg/m^2/wk i.v.	weekly	24	0	1	23
MTX	30 mg/m^2/d i.m.	daily x 3	20	0	1	19
BLEO	15 v/m^2/d i.v.	daily x 3	21	0	1	20

(Abbreviations: HMM - hexamethylmelamine; MTX - methotrexate; BLEO - bleomycin; CR - complete response; PR - partial response of >50%; NR - no response)

Thus far, HMM appears to be the only active agent in the doses and schedules employed. Nausea and vomiting were dose-limiting side effects, and little myelosuppression was noted. Two patients had sufficient responses to permit surgical resection. Of the 10 partial responders, 6 patients had squamous cell and 4 had transitional cell histology. Partial responses to trenimon (triazaquone) were noted in patients failing the above agents. Continued trials using cis-platinum and adriamycin are planned.

The following talks describing various aspects of the DCT program were given at Internal Conferences during the year by A. Goldin.

1) "Chemotherapy modalities and immune condition of the host" presented at the Symposium on Immunodepression and immunotherapy at the 10th International Congress of Chemotherapy in Zurich, Switzerland.

2) "The status of the Cooperative Monograph on US-USSR Preclinical antitumor chemotherapy test data and clinical correlations" presented at the Fourth Joint American-Soviet Meeting on the problem area "Malignant Neoplasia" held in Moscow.

3) "Preclinical contributions to the control of cancer" at a seminar on control of cancer presented at the 3rd Argentina Oncology Congress.

4) "Principles of antitumor combination chemotherapy" at the same congress.

5) "Experimental studies with anthracyclines" at the same congress.

6) "Aspects of the mechanism of action of adriamycin" at the same congress.

7) "The experimental basis of chemotherapy" presented at the Seguro Social de Peru Hospital Central No. 2 in Lima, Peru.

8) "Recent developments in the treatment of experimental solid tumors, immunochemotherapy and utilization of drug combinations in therapy" presented at the Fifth Cuban Congress on Oncology and the First with countries neighboring the Caribbean, in Havana, Cuba.

9) "The Drug Development Program of the National Cancer Institute in the USA" presented at a symposium on "Development of New Antitumor Agents" also in Havana.

10) "The cooperative aspects of the screening and drug development program of the Division of Cancer Treatment of the National Cancer Institute in Bethesda" presented at an International Seminar on Clinical Oncology in Torremolinos (Malaga) Spain.

11) "Basic Cancer Chemotherapy: Obstacles and Future Pharmacological Aspects and Selective Toxicity" prepared for the same seminar and to be published in the Proceedings (not presented because of late arrival due to snowstorm).

12) "Rationale of the chemotherapeutic adjuvant treatment" presented at an International Workshop on "Surgical Adjuvant Antineoplastic Chemotherapy in Solid Tumors" in East Berlin, German Democratic Republic.

13) "Retrospective and Prospective Approaches to Screening and to comparative Evaluation of Analogs in the USA" presented in Tokyo at the Takamatsu Symposium (A. Goldin and J. M. Venditti).

14) "Experimental models for antitumor testing in current use by the National Cancer Institute, USA: Statistical analysis and methods for selecting agents for clinical trials" (Venditti, Goldin, Miller and Rozencweig presented by A. Goldin and I. Miller at the Takamatsu Symposium in Tokyo).

15) "Preclinical toxicology of anticancer agents" presented at the Symposium on "Controversies in Cancer Treatment" at the Institut Jules Bordet in Brussels.

16) "The combined modality of chemotherapy plus immunotherapy: preclinical approaches: presented at the Third Congress at the Medical Oncology Society and Bis-Annual Meeting of the Immunology and Immunotherapy Group in Nice.

ARTICLE PUBLISHED IN A PERIODICAL:

Houchens, D. P., Johnson, R. K., Marks, T., Gaston, M.R., and Goldin, A.: Toxicity of cancer chemotherapeutic agents in athymic (nude) mice. Cancer Treatment Reports, 61, No. 1: 103-104, 1977.

Silvestrini, R., Testorelli, C., Goldin, A. and Nicolin, A.: Cell kinetics and immunogenicity of lymphoma cells treated with 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide (DTIC) in vivo. Int. J. Cancer, 19, No. 5: 664-669, 1977.

Osieka, R., Houchens, D. P., Goldin, A., Johnson, R. K.: Chemotherapy of human colon cancer xenografts in athymic nude mice. Cancer, 40, No. 5: 2640-2650, 1977.

Santoni, A., Kinney, Y. and Goldin, A.: Secondary Cytotoxic response in vitro against Moloney lymphoma cells antigenically altered by drug treatment in vivo. J. Nat. Cancer Inst., 60, No. 1: 109-112, 1978.

Testorelli, C., Franco, P., Goldin, A., Nicolin, A.: In vitro lymphocyte stimulation and the generation of cytotoxic lymphocytes with drug-induced antigenic lymphomas. Cancer Research, 38, 830-834, 1978.

Riccardi, C., Fioretti, M.C., Giampietri, A., Puccetti, P. and Goldin, A.: Growth and rejection patterns of murine lymphoma cells antigenically altered following drug treatment in vivo. Transplantation, 25, No. 2: 63-68, 1978.

Goldin, A.: Studies with high dose methotrexate-Historical Background. Cancer Treatment Reports, Vol. 62, No. 2: 307-312, 1978

Bonmassar, A., Houchens, D., Iorio, A. M., Neri, M., Santoni, A. and Goldin, A.: Soluble factors produced by normal or tumor cells, inhibiting the uptake of 5-Iodo-2'-deoxyuridine by cancer cells in vitro. European Journal of Cancer. Vol. 13, 421-430, 1978.

Riccardi, C., Fioretti, M. C., Giampietri, A., Puccetti, P., Goldin, A., and Bonmassar, E.: Growth inhibition of normal or drug-treated lymphoma cells in lethally irradiated mice. J. Natl. Cancer Inst., Vol. 60, No. 5, 1083-1090, 1978.

Putman, D. L., Kind, P. D., Goldin, A. and Kende, M.: Adoptive Immunochemotherapy of a transplantable AKR leukemia (K36). Int. J. Cancer, 21, 230-233, 1978.

Goldin, A.: The value of experimental models for prediction of clinical effectiveness. Nowotwory, Vol. 28, No. 1, 9-21, (In Polish), 1978.

Konopa, J., Privett, O. S., Jenkin, H. M., and Goldin, A.: Polysaccharides as stimulators of nonspecific immunological response against syngeneic tumors in mice. Current Chemotherapy, 1095-1097, 1978.

Goldin, A., Wodinsky, I., Merker, P. C. and Venditti, J. M.: Search for new radiation potentiators. In: Proc. of Interaction of Radiation and Antitumor Drugs, London, September, 1976. *Int. J. Radiation Oncology, Biol Physics*, 4, 25-35, 1978.

Goldin, A., Venditti, J. M., Kline, I., Wodinsky, I. and Schabel, F. M., Jr.: Preclinical investigations with ifosphamide in relation to cyclophosphamide. *Proc. of Ifosphamide Conf.* in Dusseldorf, In Press

Goldin, A.: Experimental Basis of Combination Chemotherapy. *VIIth International symposium on the Biological Characterization of Human Tumors*, Budapest, Hungary, In Press

Goldin, A.: Criteria for selection of new analogs of antitumor antibiotics. *Symposium on Antitumor Antibiotics*, May 12-13, San Francisco, California, US Japan Joint Agreement, In Press.

Goldin, A. and Kline, I.: The Bleomycins - current status and new developments in reference to experimental tumor activity. *Proc. of Symposium on the bleomycins - Current status and new developments*, In Press.

ARTICLE IN A BOOK, PAMPHLET, OR BULLETIN:

Goldin, A., Schepartz, S. A., Venditti, J. M. and DeVita, V. T., Jr.: Historical development and current strategy of the National Cancer Institute drug development program In: Methods in Cancer Research. (Eds. Busch, H. and DeVita, V. T., Jr.) Academic Press Inc., New York. In Press.

PAMPHLET OR BULLETIN ISSUED BY A DEPARTMENT OF THE UNITED STATES GOVERNMENT.

Carter, S. K. and Goldin, A.. Experimental models and their clinical correlations. In USA-USSR Monograph. Methods of Development of New Anticancer Drugs: National Cancer Institute Monograph 45. DHEW Publication No. (NIH) 76-1037, Government Printing Office, 1977.

PUBLICATIONS SECTION

Office of the Director

The Publications Section directs the preparation of Cancer Treatment Reports (CTR) (the principal scientific journal developed under the DCT Program) and provides editorial assistance to the DCT staff on a time-available basis.

CTR (formerly Cancer Chemotherapy Reports) is now in its 19th year of continuous publication. From 1959 to 1968 CTR was issued 6-10 times a year depending on the acceptance of manuscripts submitted. Several types of manuscripts were published including program information, study protocols, experimental studies, and clinical reports. Most, but not all, manuscripts were reviewed by outside referees as well as the editorial board. In 1968 the journal expanded to three parts: Part 1 (reviewed manuscripts on original research, both experimental and clinical), Part 2 (comprehensive, lengthy chemotherapy studies involving a great deal of tabular material; some of the material was reviewed by referees), and Part 3 (program information including study protocols, clinical brochures, toxicology reports, and review articles; this material was normally not reviewed). In January 1976, CTR dropped the three-part system and began monthly publication. In addition, the journal now reviews manuscripts related to all areas of cancer treatment: radiation, surgery, immunotherapy, chemotherapy, and combined modalities. All original research material is reviewed. Several issues each year now contain the proceedings of symposia or seminars.

Submissions in 1977 and 1978

During 1977 the journal received 361 research manuscripts and 61 symposia manuscripts. In the first 5 months of 1978, 198 research manuscripts and 79 symposia manuscripts were submitted.

Comparative data on the CTR series of publications are shown in table 1. manuscripts accepted are reported monthly to the Editorial Office of the Journal of the National Cancer Institute, which in turn distributes this information in their monthly reports.

Sections of the Journal

Brief Reports.--In 1973, a section entitled Brief Reports and Preliminary Communications was added to the journal. Authors were invited to submit short clinical notes or the abbreviated results of preliminary work. These reports have been processed through the editorial channels more quickly than full-length manuscripts, thus reducing costs for production time and allowing more such manuscripts to reach the readers. The response to this new section has continued to be excellent during the past year. Each issue has contained up to 10 Brief Reports and an increasing number are being submitted.

Table 1

Year	Part	Mss rec'd	Mss accepted	Issues published	Pages published	Reviewers	Total costs ($)*	Cost per page ($)
1973	1	148	112	4	524	167	23,713	54
	2	19	19					
	3	77	76	3	576		26,184	
		244	207	7	1100		59,897	
1974	1	244	138	6	978	212	42,706	40
	2	21	21	4	758		25,069	
	3	21	21	1	87		4,550	
		286	180	11	1823		72,325	
1975	1	215	114	6	1206	295	62,315	51
	2	19	19	1	263		11,374	
	3	131	138	2	419		21,809	
		365	261	9	1888		95,498	
1976		478	302	12	2021	402	89,732	45
1977		422	313	9	1771	432	ca 102,000	ca 58
1978		276**	188**	12 (scheduled)	ca 2000	--	Not available	--

*Includes the cost of printing, typesetting, and distribution only.

**From Jan 1 to June 14, 1978.

Commentaries.--In 1974, a section containing invited editorials and commentaries was initiated; in the past 2 years, many such manuscripts have been received and published. An authoritative scientist or physician is invited by a member of the Editorial Board to write briefly on a subject of current interest in cancer chemotherapy. We hope this section will encourage more interest in current topics, particularly through the submission of Letters to the Editor. In the past year we have accepted commentaries on the following topics: "Drug Interactions With Antineoplastic Agents," "Empiric Antibiotic Therapy," and "Cell Kinetics and Chemotherapy: A Critical Review."

Letters to the Editor.--During the past year *CTR* has observed a significant increase in the number of Letters submitted. These Letters usually, but not always, refer to previously published articles. In the last year a large section of each issue has been devoted to Letters.

Proceedings of Symposia

During the past several years, *CTR* has been approached frequently to publish the proceedings of scientific meetings. In 1977, the proceedings of 3 symposia were published, and in 1978/1979 we have published (or will publish) the proceedings of the following meetings:

1. Osteosarcoma Study Group Meeting
2. Conference on Noninvasive and Invasive Prospective Monitoring for Anthracycline Cardiotoxicity
3. 4th Conference on Immune Modulation and Control of Neoplasia by Adjuvant Therapy
4. Use of Steroids as Carriers of Cytotoxic Agents in Breast Cancer
5. Ovarian Cancer Mini-Symposium
6. Symposium on Cytostatic Galactitol Derivatives
7. Workshop on Relevance in Treatment of Cancer of Amino Acid Depleting Enzymes and Antimetabolites of Such Amino Acids
8. Symposium on Designs for Clinical Cancer Research

Cumulative Index

In 1975 the editorial staff began compiling a 10-year subject and author cumulative index for *CCR* Part 1 (1966-1975) and an index for all material published between 1968 and 1975 in Parts 2 and 3. Because of the extensive work required, the Publications Section contracted the completion of this work to a biologic abstractor with extensive experience in cancer research. The index has now been completed and was published in the April 1978 issue.

Official Charter for CTR

In April 1975, the first charter meeting of the *CTR* Editorial Board was held. This meeting was intended to resolve several issues concerning the organization of *CTR*, the name change to reflect the new direction of the DCT, and the additional duties of the Publications Section. In addition, the Board has written a substantive charter outlining such technicalities as the term of office for editors and the editor-in-chief (3 and 4 years respectively), the official policy regarding review and rejection, and the specific areas of research to be covered.

Editorial Board

In 1975 the Editorial Board expanded to include nine associate editors in addition to the editor-in-chief. Each year three editors will rotate off the board and three new members will be added. Provisions have been made to allow an additional year of editorship at the discretion of the editor-in-chief and the Director, DCT. In 1976, an Editorial Advisory Board with 15 members was established. The members will serve in a peripheral way to supplement the areas of expertise represented by the associate editors. Their advice regarding policy decisions, acceptance of symposia, and new areas to be covered will be solicited.

Coverage of CTR in Current Contents and Related Publications

Since 1967 CTR has been listed in Current Contents, Life Sciences. In 1973, CTR was asked to be included in a new publication of current titles in collaboration with Science, Engineering, Medical and Business Data Ltd., Oxford, England, and in 1974, we were asked to establish a similar policy with the Japan Medical Service, which publishes a supplement to its Index of Japanese Medical Periodicals listing foreign publications. It is expected that this additional coverage of the material presented in the journal will increase the demand for subscriptions, particularly in other countries.

In 1964, CTR began sending proof copies of each issue to the Chemical Abstracts Service for abstracting and indexing of the chemical information. During 1973 CTR was asked to establish a similar policy with the Biosciences Information Service (Biosis) in Philadelphia, and with Infordata International in Chicago, which publishes the Index to the Periodicals of the US Government.

Office of Management and Budget Approval

In late 1976 we submitted to the OMB our request for continuation of funds. This document outlined the purpose and scope of CTR, the publications costs incurred by the staff, a breakdown of the distribution of each issue (including the categories of subscribers), and the justification for continuing the publication. In early 1977 we received OMB approval which allows us to continue production through January 1980, at which time we will be required to request further funding.

Distribution of CTR

The Publications Section is responsible for maintaining the free mailing list for CTR. CTR is available without cost to 3225 qualified medical groups, physicians, and libraries, and is for sale to others by the Superintendent of Documents, Government Printing Office. The approximate distribution, which varies with each issue, is as follows:

3225	Foreign and domestic free mailing
1800	Superintendent of Documents (for paid subscriptions)
367	Superintendent of Documents (for Depository Libraries)
5392	

In the past several years, the number of paid subscriptions has increased tremendously. The number will undoubtedly continue to rise because of the lower limits set by the OMB each time the journal receives OMB clearance. The demand for specific back issues remains high; most requests are for the proceedings of meetings.

Problems with the service rendered by the NIH Distribution Section in maintaining the mailing list have brought about a change in the distribution procedures. Beginning in 1976 maintenance of the mailing list was transferred to the Publications Section, and is now in a computerized format. This conversion allowed more rapid service in 2 areas: (a) adding, deleting, and changing names on the list, and (b) obtaining mailing labels to be used by the contract printer in mailing the issues to the subscribers. In 1977 we screened the mailing list to eliminate addresses of subscribers who have moved, retired, or died. This clearing of the mailing list allowed us to add names of many individuals who were on a waiting list.

LITERATURE RESEARCH SECTION

Office of the Director

The Literature Research Section serves the Division of Cancer Treatment by providing information from published literature on all aspects of the therapy of cancer. Data from the fields of chemotherapy, radiotherapy, surgery, immunotherapy and the related chemical and biomedical disciplines are used by the staff in Decision Network review, meeting FDA requirements for IND filing, preparing clinical brochures, and as background for evaluation of toxicological and clinical studies.

Literature Services. The Section received and filled over 300 requests for information during the year. Responses were provided as comprehensive or selected bibliographies, computer print-outs, abstracts and copies of articles. More than half of the requests entailed manual literature searches supplemented by the various automated bibliographic retrieval systems such as Medline, Toxline and Cancerline. Extensive clinical searches were done for the use of high-dose methotrexate, intrathecal administration of antineoplastic agents, manifestations of toxicity of chemotherapeutic drugs, adjuvant therapy of various neoplasms and immunotherapy of lung cancer. Bibliographies were prepared, or updated, for such agents as PALA, misonidazole, the epipodophyllotoxins, sugar alcohols, adriamycin, imidazopyrazole, methyl melamines, aphidicolin, macromomycins and cis-Pt. Searches were also done for various enzyme systems, reactive nitrogen mustard intermediates and the use of heroin in terminal cancer patients.

The Section co-ordinates NCI access to the on-line data bases of the Medlars automated bibliographic search system. In this role the Section serves all areas of the Institute, processing searches and providing assistance and instruction in the use of the system. It is also the center for access to the Toxline and Cancerline data bases for personnel in the Blair Building. These computer services aid in the searching and facilitate the answering of requests. Responses to more than 70 requests were in the form of computer print-outs only. Monthly SDI (Selective Dissemination of Information) bibliographies are produced for members of the staff on specific subjects of continuing interest.

The staff continues to be responsible for the preparation and distribution of the agent folders for Decision Network meetings. Each folder contains the structure and systematic name of the compound, a bibliography from the published literature, summaries of screening, toxicological or other preclinical or clinical data, and questionnaires. Materials were assembled for a total of 32 drugs for the three meetings held this year.

A further responsibility of the Section is the maintenance of the Cancer Therapy Library, a collection of books and journals for the use of NCI staff. Copies of over 60 journals are regularly received including many of the cancer journals, abstracting and indexing secondary sources, and chemical, biomedical or information science journals of special interest to the Section and other personnel in the building. Approximately $12,000 was spent on books and subscriptions.

Contract Operations. Contracts for the preparation of Cancer Chemotherapy Abstracts and its successor Cancer Therapy Abstracts have been monitored by members of the Literature Research Section since 1965. In addition to the printed journal and hard copy of all cited articles, computer tapes of the abstracts are received from the contractor. Under the sponsorship of the International Cancer Research Data Bank, these are input to the Cancerline file which can be searched on-line through the Medlars network.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1977 - September 30, 1978

The Developmental Therapeutics Program (DTP) has the major responsibility for the preclinical drug development and related activities for the Division of Cancer Treatment. The extramural activities of DTP are supported by contracts, CREGs and grants. These are administered and evaluated scientifically by four collaborative branches located in Silver Spring, Maryland. These include the Drug Synthesis and Chemistry Branch, Natural Products Branch, Drug Evaluation Branch, and Pharmaceutical Resources Branch.

The intramural research program, whose activities are concerned primarily with the design, synthesis, evaluation and mechanism of action of antitumor agents, the pharmacological and toxicologic properties of drugs at the molecular, cellular, and whole body levels, and the molecular biological aspects of neoplastic transformations, consists of five intramural laboratories located on the National Institutes of Health campus in Bethesda, Maryland. The laboratories include the Laboratory of Medicinal Chemistry and Biology, Laboratory of Toxicology, Laboratory of Chemical Pharmacology, Laboratory of Molecular Pharmacology, and Laboratory of Tumor Cell Biology. The administrative leadership and scientific integration of the total DTP are centralized in the Office of the Associate Director.

Program Accomplishments

Decision Network Actions

Decision Network (DN) Committee meetings during this reporting period resulted in the assignment of high-priority status to the following agents which have not yet passed DN-4 (Phase I clinical trials):

PALA (Phosphonoacetyl-L-aspartate, NSC-224131)

- Stage IV - in Phase I clinical trials

Pentamethylmelamine (NSC-118742)

- Passed DN3 (toxicity established in animal models, ready for Phase I clinical trials)

AD-32 (Adriamycin derivative, NSC-246131)

- Stage IV - in Phase I clinical trials

Bruceantin (NSC-165563)

- Stage IV - in Phase I clinical trials

m-AMSA (Acridine derivative, NSC-249992)

- Stage IV - in Phase I clinical trials

2'-Deoxycoformycin (Deaminase inhibitor, NSC-218321)

- Passed DN3 as single agent. Currently in large animal toxicological evaluation (Stage III) in combination with arabinosyl adenine-5'-monophosphate (NSC-259272).

AZQ (Aziridinylbenzoquinone derivative, NSC-182986)

- Stage III - in large animal toxicology studies

5-Methyltetrahydrohomofolate (NSC-139490)

- Passed DN3, IND in preparation

Dichloromethotrexate (NSC-29630)

- Stage III - in large animal toxicology studies as high dose agent with citrovorum rescue.

During the past year, the DN Committee continued to reassess the priorities of new agents in the drug development pipeline. This led to several agents being dropped from further consideration as candidate antitumor agents. As of June 1, 1978, 52 agents with diverse chemical structures remain in various stages of drug development prior to Phase II clinical evaluation (Stage V) for activity in the "signal tumors."

Acquisition of Agents for Screening

Mainly through the efforts of the Drug Synthesis and Chemistry Branch and the Natural Products Branch, 14,942 pure crystalline substances were submitted to the Program during the past year for primary screening. Synthetic products accounted for 14,490 of these submissions and the remaining materials were of fermentation, plant and animal origin (Table 1). Confirmed activity was seen in 10.8% of the total submissions of crystalline materials and represented an increase in actives from 7.2% in 1976. This increase was attributed to the use of a refined preselection technique for compounds for the primary screen which had the added benefit of providing some new classes of chemicals. The major source of crystalline compounds came from voluntary submissions by industry but the largest percentage of active compounds were derived from direct purchases (29%), contract-supported laboratories (28%) and government laboratories (18%). Sixteen new compounds passed DN2A (Table 2). These included three fermentation products, two plant products, and 11 synthetic compounds.

Primary Screening and Secondary Evaluation

The Drug Evaluation Branch reported that the past year brought an increase of 15% in panel testing over the previous year despite a 30% reduction of compound input to the primary screen. The projected 1,000 compounds per year tumor panel testing capacity was reached for the following mouse tumor models: B16 melanoma, colon tumor 38, leukemia L1210, and the Lewis lung carcinoma. Approximately 750 compounds per year can be screened against the $CD8F_1$ mouse mammary carcinoma, and 375 compounds per year against the human mammary, colon and lung xenografts. Animal disease problems in both the breeding and testing laboratories employing the "nude" mouse have hampered the desired screening capacity in the xenografts but this situation is expected to be rectified during the next year.

Studies on the histologic, biochemical and growth characteristics of various human tumor xenografts continued to be an important contract-supported activity as well as the testing of the sensitivity of these tumors to known clinically effective drugs. Various patterns of activity were seen, but the most significant finding was that individual human tumors of the same histological type differ in response to the same drug. This emphasizes further the need for the development of a rapid *in vivo* test to determine differences in sensitivities between the same tumor types to various antitumor agents. The kidney capsule test under contract-supported development is a candidate for such a rapid assay system which will be further explored by the Program during the coming year. Several new human tumor xenografts, originating from ovarian and renal carcinomas, and a transplanted mouse ovarian carcinoma are under contract-supported evaluation and appear as possible new candidates for the tumor panel.

Production, Formulation and Pre-clinical Toxicology

The Pharmaceutical Resources Branch continued to provide sufficient quantities of bulk chemicals and radio-labeled and formulated drugs for laboratory investigations, preclinical large animal toxicologic studies, and clinical trials. Of particular note was the production and formulation of high-quality methotrexate for high-dose studies which resulted in a considerable financial savings to the Division. An updated computerized inventory system of all clinical products was implemented during the past year and is now fully operational. This system provides proper drug distribution to numerous authorized clinical investigators and programs and promptly answers queries about drug cost, shipment information, distribution trends, etc. Successful formulations were developed by contract supported laboratories for such high-priority agents as PALA (NSC-224131), pentamethylmelamine (NSC-118742), 5-methyltetrahydrohomofolate (NSC-139490) as well as for DON (NSC-7365), 6-selenoguanosine (NSC-137679) and others. Another major contribution of the Branch was the identification, evaluation, and quality assay of various Mexican laetrile products. This was done to assist the Division and the Institute in their assessment of the biological activity of this material.

The Division, as well as the NIH, benefited during the past year from the contract-supported activities of the Chemotherapy Fementation Laboratory operated at the Frederick Cancer Research Center. Over 800 grams of high-quality daunorubicin were prepared by a streptomycete fermentation for use in NCI sponsored clinical trials and at a substantial financial savings to the Division. In addition, increased production of human lymphoblastoid interferon was accomplished to provide a regular supply of this biologic response modifier to Dr. C. B. Anfinsen's (NIAMDD) research program at the NIH in sufficient quantities for further purification and characterization. Piperazinedione (NSC-135758), an antitumor agent with clinical promise, was also successfully prepared in small quantities in preparation for further scale-up. This agent was initially available from a commercial source which subsequently discontinued commitment of its resources for further production of this compound for NCI clinical trials.

In the pre-clinical toxicology area, full protocol studies (Task I) were completed through the Prime contract mechanism on PALA (NSC-224131), pentamethylmelamine (NSC-118742), 5-methyltetrahydrohomofolate (NSC-139490) and 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea, PCNU (NSC-95466). Of particular note was the ability of the Prime contract to respond to the Program's request to conduct large animal protocol toxicologic studies on PALA at an accelerated pace in order to move this high-priority agent towards Phase I clinical trials as quickly as possible. This was accomplished as evidenced by the submission of a final toxicologic report to the Program in a record 4-1/2 months following initiation of the studies. In addition to many abbreviated/special studies completed during the past year, the Prime contract fully implemented an animal model screen for nephrotoxicity. Platinum containing analogs of the clinically important -diamminedichloroplatinum (NSC-119875) are being evaluated to determine whether they are less nephrotoxic than the parent drug.

Biologic Markers Program

The Biological Markers Laboratory (BML), operated under contract at Frederick Cancer Research Center (FCRC), has been fully staffed and operational this past year and now constitutes the largest part of DTP's biologic markers program. Its work this year included discovery of a new malignancy-associated protein (designated MAD-2), continuing investigation and methods development for the C3DP malignancy-associated protein, for calcitonin, for cysteine aminopeptidase, for plasminogen activators, and for hCG. As part of the foregoing, a number of peptides were synthesized, antigens were purified and antisera for RIA were produced. Over 50 human cell lines have been studied for marker production *in vitro* in an investigation of factors leading to oncofetal gene expression. Other projects involved cell membrane markers, a search for a reliable pancreatic carcinoma marker, quantitative studies of tumor cell and marker kinetics and collaborative clinical evaluations of new and old markers with NCI and Johns Hopkins patients.

The clinical investigations of markers involved NCI (ovarian, testicular, lung and breast), NCI sponsored cooperative clinical groups (gastrointestinal, lung), Johns Hopkins (small cell lung, breast, ovarian, colon), and University of Kentucky (leukemia, lymphomas). Analytical work has been performed by

FCRC, University of Kentucky, University of Missouri, University of Colorado, and Oak Ridge National Laboratory.

An ad hoc scientific advisory group was organized this year. It held two meetings to review DTP's markers program and to review the field of biologic markers research generally. The objective was to identify topics for DTP to pursue or discontinue.

Highlights of Intramural Laboratory Contributions

1. The Laboratory of Chemical Pharmacology (LCP) developed analytical assay techniques for PALA (NSC-224131), m-AMSA (NSC-239992), cis-diamminedichloro platinum (NSC-119875), misonidazole (NSC-261037), and thymidine (NSC-21548) to assist laboratory investigators and clinicians in the pharmacologic and clinical evaluation of these agents.

2. The Laboratory of Toxicology (LT) initiated studies on the enzymologic and pharmacologic basis for resistance of certain tumors to PALA (NSC-224131), an agent which acts by restricting the availability of pyrimidines through its inhibition of L-aspartic acid transcarbamylase (ATCase). The levels of this enzyme were measured in various mouse tumors, and were shown to correlate with sensitivity to PALA. These studies are being extended to cancer patients in an effort to determine the sensitivity of individual malignancies to PALA treatment.

3. An enzyme inhibition titration assay method was developed by the Laboratory of Medicinal Chemistry and Biology (LMCB) which measures levels of 2'-deoxycoformycin (NSC-218321) in biological fluids at drug concentrations in the nanomolar range. The assay has made possible pharmacological studies of this agent in experimental animals and man where it is being used as a lympholytic agent and as a deaminase inhibitor for arabinosyl adenine monophosphate (NSC-259272).

4. In continuing its studies on repair of DNA damage by various modifications of its alkaline elution technique, the Laboratory of Molecular Pharmacology (LMP) reported that Adriamycin (NSC-123127) produces single strand breaks in mammalian cells grown in culture but in addition, induces formation of DNA-protein crosslinks at the break sties. This latter unexpected effect was also demonstrated with other intercalating agents such as ellipticine (NSC-71795), actinomycin (NSC-3053), ethidium and lucanthone, but not with agents which bind to DNA without intercalation or which inhibit DNA synthesis without binding. The frequency of this DNA lesion however did not appear to be related to the cell-killing potential of the drug but was instead thought to be a physiologic response to changes in DNA coiling.

5. The continuously replicating leukocyte line (HL-60) from the blood of a patient with acute promyelocytic leukemia, first established by the Laboratory of Tumor Cell Biology (LTCB) over a year ago, has maintained its myeloid markers. This cell line undergoes differentiation in the presence of DMSO analogously to the differentiation of the Friend mouse erythroleukemia cells in DMSO by the appearance of granulocytes. The HL-60 cells are also capable of infection with primate type-C RNA tumor viruses resulting in complete loss

of the myeloid markers and differentiation in the presence of DMSO. This culture system is currently being investigated as a rapid in vitro assay for chemotherapeutic agents which can selectively exert their cytotoxic effects against this leukemia.

6. The LT has shown that continuous subcutaneous infusion of bleomycin to Lewis lung carcinoma-bearing mice is superior to intermittent injection by the same route as evidenced by a reduction in pulmonary toxicity and an increase in therapeutic efficacy. This represents a spin-off of the Laboratory's efforts to develop an animal model for assessing the pneumotoxicity of bleomycin analogs and suggests similar use of the drug in the clinic.

7. LCP investigators have developed a modification of the Ames test for detecting the mutagenic effects of chemical compounds. The assay allows detection of the entire set of possible mutagenic lesions in DNA caused by chemical compounds and several antitumor agents are currently being examined in this system.

8. In extending studies on nucleosides related to the clinically active 5-azacytidine (5-AC, NSC-102816), the LMCB reported the first successful synthesis of the derivative, arabinofuranosyl-5-azacytosine (ara-5-AC). This compound shows activity comparable to 5-AC in L1210 leukemia in vivo and is undergoing studies to determine whether it is biochemically similar to ara-C and 5-AC with regard to deamination and nucleotide formation.

9. The LTCB reported that a significantly high percentage of patients in acute blast phase of CML who are positive for the enzyme terminal deoxynucleotidyl transferase show a response to vincristine-prednisone therapy.

10. A new quantitative autoradiographic technique was developed by LCP investigators which employs radioactive-labeled non-metabolizable α-aminoisobutyric acid to measure up to a 100-fold increase in capillary permeability in focal brain regions 100 microns in diameter. The technique allows quantitation of defects in the permeability of the blood brain barrier caused by various disease states.

11. In pursuing its interest in developing organ-specific antitumor agents, the LMCB, which has already contributed AZQ (NSC-182986) and spirohydantoin mustard (NSC-172112) to the Program as potential CNS antitumor agents, synthesized propranolol mustard as an antitumor agent with potential for localization in lung tissue.

12. LT studies on the enzymologic basis for the toxicity of L-alanosine (NSC-153353) to tumor cells and normal tissues are linked to its inhibition of adenylosuccinic acid synthetase through condensation in vivo with a fragment of the purine aminoimidazole carboxylic acid ribotide. The adduct is susceptable to destruction by alkaline phosphatase, 5'-nucleotidase and adenylosuccinic lyase. Measurement of the specific activities of these enzymes in various tumors and normal tissues may reveal the biochemical basis for sensitivity to the drug.

13. Using restriction enzyme digestion, the LTCB investigators have obtained "clips" of human DNA's serived from normal and leukemic tissues which can hybridize to RNA from primate type-C RNA tumor viruses. Related DNA sequences are similarly distributed in both virus negative normal and tumor tissues of cats which can hybridize to the feline leukemia virus. The latter system might prove useful in the detection of possible oncogenic viruses in man.

14. Initial studies by the LCP on the development of pharmacologically active polymers for improved cancer treatment have resulted in the preparation of methotrexate (MTX) linked covalently to divinylethermaleic anhydride copolymer (DIVEMA). The polymer was evaluated against the L1210 ascitic leukemia and the Lewis lung subcutaneous carcinoma. In each case the MTX-DIVEMA copolymer was significantly more active than methotrexate or DIVEMA alone at optimal dose and schedule. Higher doses of MTX-DIVEMA copolymer altered the pharmacokinetics of MTX and increased toxicity whereas higher doses of DIVEMA alone were without enhanced toxicity.

15. As a means of assessing the pharmacological action of nucleoside anticancer agents, the LMCB has studied the effects of cordycepin (3'-deoxyadenosine) and 9-β-D-xylofuranosyladenine (XA) on the synthesis and processing of RNA in the presence and absence of the adenosine deaminase inhibitor 2'-deoxycoformycin (2'-DCF, NSC-218321). The potentiation by 2'-DCF of the cytotoxic and antitumor effects of cordycepin and XA in vivo suggest a relationship to inhibition of nuclear RNA synthesis.

TABLE 1

MATERIAL ACCESSIONED AND ACTIVITY REPORT (1977-8)*

	Crude Extracts		Crystalline Materials	
	No. submitted	No. active	No. Submitted	Confirmed activity
Fermentation Products	1347	138	205	29
Animal Products	227	52	62	1
Plant Products	6153	807	185	19
Synthetics	-	-	14490	1566
	7727	997	14942	1615

*April 1977 - March 1978

TABLE 2

COMPOUNDS PASSING DECISION NETWORK 2A

NSC No.		
21548	Thymidine	Synthetic
118742	Pentamethylmelamine, hydrochloride	Synthetic
125973	Taxol	Plant
196473	9,10-Anthracenedione, 1,4-bis[[2-[(2-hydroxyethyl) amino]ethyl]amino]-	Synthetic
201628	Carbonic dihydrazide, bis[(3,5-dibromo-2-hydroxyphenyl)methylene]-	Synthetic
237020	Largomycin	Fermentation
234714	Aphidicholin	Fermentation
259272	9H-Purin-6-amine, 9-(5-O-phosphono-β-D-arabinofuranosyl)-, disodium salt	Synthetic
259968	Bouvardin	Plant
261036	1,2-Propanediol, 3-(2-nitro-1H-imidazol-1-yl)- (RO-5-9936)	Synthetic
261037	Misonidazole (RO-7-0582)	Synthetic
282175	Androst-4-ene-3,17-dione, 4-hydroxy-	Synthetic
283162	Trimethyltrimethylomelamine	Synthetic
288411	Indenoquinoline carboxylic acid, sodium salt	Synthetic
296961	Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester) (WR-2721)	Synthetic
526417	Echinomycin (Quinomycin A)	Fermentation

SUMMARY REPORT OF THE DRUG SYNTHESIS AND CHEMISTRY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT
October 1, 1977 - September 30, 1978

Since cancer is not a single disease but a number of diseases affecting the different organs and systems of the body, several approaches to cancer treatment may be selected. The major approaches include surgery, radiation, chemotherapy, immunotherapy, or a combination of the four. Research in drug development includes selection and procurement of agents for testing, bioassay of the agent in tumor systems, preclinical pharmacological and toxicological study, and clinical evaluations. The foundation of any chemotherapy program is the drug itself - either single or in drug combination. Consequently, the search for more effective, less toxic and broader acting agents is the basic step in all chemotherapy programs.

Past experience has clearly demonstrated that effective antitumor agents are not restricted to a single, or even a few chemical classes but that the chemical-structural parameters are broad. One must conclude then that all effective agents are not operating by a single or the same mechanism. Therefore, efforts are directed towards the chemical modifications of existing drugs to increase their activity and/or decrease their toxicity, and to search for new agents via chemical synthesis, or from natural sources. The development of second generation analogs is of prime interest to improving existing leads. Materials will be evaluated in the primary screen and those agents demonstrating sufficient activity will be subjected to toxicological studies and possible clinical evaluation.

Research and development efforts are, in large measure, based on leads provided by the primary screens, biochemical and toxicological studies, pharmacokinetics observations, literature reports, and the surveillance of the worldwide scientific community for information on new drugs. As the vast storehouse of information accumulates and greater achievement is being experienced in the control of the more rapidly proliferating phase of cell growth, the search for new structures aimed against the slower growing tumors in specific organs, i.e., lung, breast, colon, brain, etc. will be the prime objectives.

Branch Staff

The Drug Synthesis and Chemistry Branch is composed of a staff of persons consisting of five senior professional scientists (GS-13 and above), six other professional staff members (GS-9 to GS-12) and the remainder (8) technical and clerical staff.

Those responsible for the activity of the two Sections are as follows:

Drug Synthesis & Chemistry Branch
Chief -- Dr. Harry B. Wood, Jr.

Drug Synthesis Section
Head, Dr. Venkatachala L. Narayanan

Chemical & Drug Information Section
Mr. Sidney Richman

The Acquisition of Chemicals Unit (Head, Mr. Robert B. Ing) operates as an activity from the Office of the Chief of the Drug Synthesis & Chemistry Branch.

Objectives

As part of the broad Division of Cancer Treatment Program, the Drug Synthesis and Chemistry Branch has three major objectives:

I. To develop new drugs via a chemical contract synthesis program.

II. To acquire new agents for screening via acquisition from the scientific community-at-large.

III. To develop, maintain and utilize a chemical information system.

Implementation of Program

These objectives are carried out through the activities of the Branch's Sections which are:

I. The Drug Synthesis Section: The objectives are divided into the design and synthesis of new drugs. The thrust is directed towards analog improvement, i.e., broader spectrum of activity, and decreased toxicity, and the development of new exploratory leads and the synthesis of unique structures as potential anticancer agents and second generation analogs.

Even though major advances have been made in recent years, the treatment of cancer is still limited by the availability of useful agents. The usefulness of the majority of the present agents are limited also by severe toxic side effects and/or associated with the development of drug resistant tumors. Therefore, a greater variety of agents with diverse structures and different mechanism of action, broader spectrum of activity and a greater safety would be extremely valuable in cancer treatment. The problem of finding these improved drugs is the main objective of the contract synthesis program and its efforts are channeled into many areas of investigation. As the rapidly growing tumors become more and more under control, attention is being focused upon developing new drugs for the slower growing tumors, especially for lung, breast, colon, etc.

II. Chemical and Drug Information Section: The objective of the Section is to develop and operate a data processing system and to devise and use various computerized techniques to meet the scientific, operational control and management planning needs of the Branch. The Section is concerned with: (1) operating, maintaining, and enhancing the automated Drug Synthesis and Chemistry's Chemical Information System which supports the accessioning, storage, and distribution of chemicals and drugs acquired for antitumor testing, including the coordination of staff and contract activities related to CIS; (2) managing the storage and retrieval of chemical information in both automated and manual DTP files; (3) conducting structure-activity studies to optimize acquisitions by identifying chemical classes to be deemphasized (because they are largely negative or adequately studied) and by selecting new accessions from among potential acquisitions, aided by a developing model which assigns activity scores in the P388 lymphocytic leukemia prescreen to such compounds; and (4) identifying for acquisition compounds of interest which are reported in the literature or are in various chemical files maintained by governmental agencies, industrial organizations, and information venders.

III. Acquisition of Chemicals (Office of the Chief): The objectives of this effort are to insure the continuous flow of approximately 15,000 new preselected synthetic compounds annually for the primary screen and to acquire approximately 500 to 1000 samples for the tumor panel. Extensive efforts are maintained with industry, government laboratories, and with the scientific public in academic and research institutions.

Effort is directed toward (1) developing and maintaining sources for preselected compounds with unique structural features and biological activity; (2) the acquisition of compounds for the primary screen and tumor panel evaluations; (3) developing compound selection criteria; (4) conducting structure-activity correlation studies; (5) monitoring the discreet agreements with industrial suppliers; and (6) providing for the proper storage and distribution of all accessioned materials.

Operation of Program

The first full year of selective acquisition began in 1977. Methodology implementing the criteria for selection has been effectively established and the preselection process is operational. The use of criteria codes that is assigned to each compound provides the necessary feedback mechanism on screening data summaries which permits the analysis for effectiveness of selection criteria and for structure-activity correlation studies. During the 1977 calendar year a total of 15,160 preselected compounds were assigned NSC numbers. The following table provides an overview of the results from preselection.

TABLE I

EFFECT OF PRE-SELECTION ON COMPOUND ACQUISITION

TUMOR SYSTEMS	NO. CONFIRMED ACTIVES 1976	NO. CONFIRMED ACTIVES 1977
B16	6	5
GL	-	2
LE	175	117
LL	2	8
P388	822	1467
WA	22	30
9K	50	50
Total	1077	1679

A review of the P388 actives indicate that a large number of these actives have low T/C values and do not warrant evaluation in the panel. Many of these active leads are structurally unique and their structural features have been coded and incorporated into selection criteria. Concerted effort is made to procure analogs and homologs of these lead compounds. As a result of these follow-up procurement efforts compounds with improved activity have been uncovered and are currently assigned for panel testing.

Since July 1976, a total of 947 compounds have been assigned for testing in tumor panel.

TABLE II

PROFILE AND CURRENT STATUS FOR ACQUISITION OF PANEL COMPOUNDS

ADEQUATE SUPPLY		
1. DN2, By-pass, special studies	209	
2. Assigned from primary screen	87	
3. Purchased	53	
4. Prepared by Prep Labs	78	427
PROCUREMENT EFFORTS REQUIRED		
1. Natural Products (Procured by Natural Products Branch)	102	102
2. Synthetics		
Purchase	0	
Assigned to Prep Labs	101	
Industrial	121	
Non-Industrial	24	
Contracts	12	
USSR	5	
QNS (Awaiting response from supplier)	155	418
Total number of panel compounds assigned		947

The Drug Synthesis and Chemistry Branch has been successful in providing sufficient samples of 427 compounds for panel testing. There are 418 compounds which are QNS of which 101 are being synthesized by the prep labs. At the present time approximately 175 synthetic compounds being evaluated in the panel are the direct result of preselection. Four compounds are in DN2A (NSC-279836, NSC-287513, NSC-201628 and NSC-288411). Active lead compounds in some of the slower growing tumors have been identified and effort is being made to analyze their structural characteristics that could be applied in the acquisition process. As panel testings are completed, additional compounds with sufficient broad spectrum activity will be presented for Decision Network consideration. There is an overall increase in the total numbers of actives from 7.2% in 1976 to 10.92% in 1977 and an increase from 1.5% to 10.92% for the past two years period (1975-1977).

To arrive at the annual input of 15,000 selected compounds, the acquisition team must examine structures of over 50,000 compounds. It is imperative, therefore, that sources for a large reservoir of compounds be maintained. This is accomplished through liaison efforts with major industrial sources. After twenty years of continuous sampling from domestic industrial sources, there are indications which suggest that we no longer can depend on some of our prime domestic pharmaceutical companies to sustain the NCI with compounds. The following table is an analysis of the contributions made by some of the major domestic pharmaceutical suppliers.

TABLE III

COMPOUNDS CONTRIBUTED FROM U.S. PHARMACEUTICAL COMPANIES

COMPANY	COMPOUNDS PER YEAR				
	1973	1974	1975	1976	1977
Abbott Laboratories	930	2435	1278	911	913
Bristol Laboratories	1	1	0	6	3
Burroughs-Wellcome	0	0	0	0	1
Ciba-Geigy	8	1	0	0	11
Hoffmann La-Roche	50	27	50	19	44
Lederle (American Cyanamid)	3	1	0	0	0
Eli Lilly	0	0	0	46	12
McNeil Laboratories	305	3	0	140	0
Mead Johnson	1	0	0	133	5
Merck Sharp & Dohme	3	11	250	0	393
Merrell-National	73	8	4	28	43
Miles Laboratories	1881	17	5	5	0
Norwich Pharmaceutical	681	137	0	0	52
Parke-Davis	1913	2421	197	57	19
Pfizer	46	0	24	0	12
Riker Laboratories	0	0	0	0	90

COMPANY	COMPOUNDS PER YEAR				
	1973	1974	1975	1976	1977
Sandoz Pharmaceutical	903	839	936	899	15
Schering Corporation	195	102	117	67	61
Searle Laboratories	1	1	4	2	12
Smith Kline & French	11	179	0	0	160
Squibb	36	131	0	0	32
Sterling-Winthrop	95	38	2	13	17
Upjohn	18	462	117	98	12
Wyeth Laboratories	0	1	0	1	9
Total	7150	6830	3050	2140	1910
Total NSC Nos. assigned	34430	39690	21410	15710	15160
% contribution	20.6	17.2	14.2	15.5	12.6

To counteract the diminishing supply for U.S. sources, the acquisition team has initiated efforts to develop new sources through direct liaison with other foreign chemical and pharmaceutical houses and by stepped-up correspondence with chemists on a worldwide basis. The acquisition effort will be restricted in the coming year due to budgetary constraints incurred.

One major thrust of second generation analog synthesis will be in the area of clinically effective drugs not being pursued by industrial concerns or those supplied via contracts. Analogs will be designed for therapy improvement and decreasing the undesirable toxic features of the parent drug. Also analog synthesis will be aimed at improving those agents which have shown therapeutic effectiveness in the screen but have been abandoned later because of toxicological or other problems. Synthetic analogs of known active drugs may possess superior properties because of better transport and greater resistance to degradation or greater binding to appropriate enzyme sites. Synthetic analogs may produce longer or shorter duration of activity as the needs dictate, alter the solubility characteristics and improve the stability. Structural modifications could also introduce new chromophore groups to aid in determining the drug level in the host. Analogs would also be used in mechanistic studies, and biochemical investigations which include areas of whole body drug distribution, drug metabolites, enzymatic activity, and the route and rate of excretion.

New chemical structures or analogs, developed through biochemical cell cycle kinetic studies, pharmacological and pharmaceutical rationales, and biological activities demonstrated in non-cancer program, should provide additional input to the directed preselection and synthesis approach. Plans are underway to group together those agents which have shown no cross-resistance and determine if there are any structural similarities for future analog design. The same study will be made on those agents which have shown potentiations in classical biological and/or biochemical screens.

The major synthetic activity will be towards broader acting drugs. The search for agents which have a high degree of organ localization will be undertaken and compounds containing this property should be considered as potential leads for chemical modification. A program to synthesize drugs localizing in the lung, breast, colon, etc. will be considered and their chemical structure will be modified with the objective of introducing antitumor properties. Organ specific screens are needed to evaluate such new types of agents (specific toxicological screens, renal, cardiotoxic, neurotoxic, etc). The increasing awareness that many antitumor drugs may possess latent carcinogenic properties will be considered in the hopes of eliminating such characteristics. Synthetic development may provide improved methods of synthesis which would achieve economic advantages in the production of large amounts of drugs for the clinic, i.e., methotrexate during this past period.

Chemical modification and structural analogs of natural products will also provide unique compounds of interest. The continued follow-up of empirical observations and changes in the structure of the already effective agents will also add to our array of new material for investigations.

The Branch is keenly interested in new approaches to drug design. One such approach being exploited is the synthesis of analogs to develop and exploit structure-activity relationships. When a significant new activity has been established, there is need for a major effort to prepare and study related derivatives for the purpose of defining the relation of structure to biological activity. It is desirable to study a series of closely related active compounds to determine toxicity, transport, and other chemo-physical properties. Recognizing that such techniques are still in the early stages of development, effort will continue to improve and develop new methodologies. Several problems do still exist in this area of investigation; such as obtaining the physical-chemical measurements which are quite exact whereas the biological testing data has a considerable amount of variation. It must be recognized that this approach may not necessarily give immediate answers since it may be necessary to develop new parameters. However, it is hoped that such studies will focus attention on synthetic areas of greatest potential and provide the chemist with more directed synthetic approaches. This is not to be interpreted that new structural classes will be by-passed.

Worldwide Surveillance

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries, grantees and academic institutions, research organizations and other governmental laboratories, since s substantial number of compounds are contributed to the Program on a voluntary basis from these sources. Good public relations must be and have been maintained by reporting of data and by making special handling of unusual agents a matter of normal service. With the increasing complexity of data processing and growth of the Program, constant awareness of the problems of 'Commercial Discreet' data must ever be present. A total of 4337 individual suppliers, of which 1687 are industrial suppliers, have contributed samples to the Program. During the reporting period there have been 371 new non-industrial suppliers and

159 new industrial suppliers submitting materials for primary screening. Even though the majority of these contributors are domestic suppliers many materials have been supplied from foreign countries. The increasing number of contributors is an index of the continued enthusiasm for the Program by the scientific community.

The Branch's continuous international surveillance for acquiring new agents, synthetic and natural products, has provided over 1000 foreign suppliers from industry, academic institutions and governmental and research organizations. Close contact is maintained with these and other potential new suppliers to provide interchange of chemical data and biological information on potential chemicals and drugs of interest to the NCI and the scientific community-at-large.

The Branch has 20 contracts; 17 synthesis contracts, see Table IX under the Drug Synthesis Section; 1 chemical information contract, see Table X under the Chemical Drug Information Section; and 2 miscellaneous contracts, see Table IV below under Miscellaneous Branch Contracts.

TABLE IV

MISCELLANEOUS BRANCH CONTRACTS

CONTRACTOR	INVESTIGATOR	CONTRACT NUMBER
Microbiological Associates, Inc.	Mozejko	NO1-CM-43793
Starks C.P., Inc.	Paull	NO1-CM-53745

The Starks C.P., Inc. activities includes: (1) selective acquisition of novel chemicals and drugs for the primary screen, (3) serves in a liaison capacity to develop new sources of compounds, (3) monitors chemical literature for unique structures of interest, (4) prepares the necessary documentation for the computer registration of samples, (5) monitors the flow of computer generated out-puts to suppliers and to management, (6) maintains accurate records of all transactions dealing with liaison activities with suppliers, the operation contractor for the Chemical Information System and with the contractor for storage and distribution of chemicals.

Microbiological Associates, Inc., provides the Division of Cancer Treatment with services for the storage of bulk chemicals and for the distribution of these materials to program research laboratories throughout the world. The contractor also maintains an inventory and shipping record for these bulk materials. Custody and records of the 'Drug Abuse' materials are also maintained.

New Areas of Investigation

I. Development of Radiosensitizers

The treatment of solid tumors via radiation does not always eradicate the total local tumor cell population. It is recognized that the inner core of solid tumor mass has a decreased blood supply and consequently a decreased oxygen supply. Cells deficient in oxygen content (hypoxic cells) are able to resist the killing affect of radiation. Some classes of chemicals, those with electron affinity, appear to mimic the effect of oxygen in the cell. The resistance may be decreased by the use of drugs that can sensitize the cells to radiation and assist in eliminating the tumor burden. Therefore a contract effort is being developed with the objective of synthesizing compounds which will (a) diffuse readily into tumor cells, especially cells with a poor blood supply, (b) metabolize slowly to non-toxic metabolites, and (c) sensitize cells to radiation but not inhibit the repair mechanism of normal cells. Many of the compounds, i.e., nitroimidazoles, possess neuro toxicity and it is hoped that the new generation of compounds in this class will have less toxic affects. Preliminary studies suggest several types of compounds as potential candidates. As radiosensitizers those include nitroimidazoles, nitrothiazoles, nitropyrimidines, thiosugars, etc. Such compounds will be evaluated for electron affinity, partition coefficient, *in vitro* and *in vivo* effects upon radiation of tumors with hypoxic cells.

II. Development of Methods for Preselection of Compounds for the Preliminary Screen

The study and analysis of the chemical file combined with broad structure activity relationship is providing valuable guides for preselection compounds for the screen. During this past report period these guides have increased the number of actives from 7.2% to 10.92% and an increase from 1.5% to 10.92% over the past two years. This is a major improvement and as the methodology is refined there is every reason to expect this rate to increase. Fortunately this increase has also provided some new classes of chemicals. Some of the new guides include: (a) deemphasize compounds with less than 12 carbons with only one functional type, (b) select compounds with quternary nitrogen, (c) select compounds with phosphonium ions, (d) select compounds possessing the "ABONS" criteria, i.e., aromatic benzylic group with an electron rich substituents and with an oxygen, nitrogen, or sulfur in alpha position of the side chain, (e) deemphasize hydrocarbons, (f) deemphasize poly halogen aliphatic and aromatic compounds.

Accomplishments

The Drug Synthesis and Chemistry Branch provided a steady flow of synthetic materials into the Program. Other crystalline substances (see Table V) from plant products, marine and insect products and fermentation sources were furnished by the Natural Products Branch. Some of these materials have demonstrated confirmed activity in one or more of the primary screens (Step III) and have been assigned as candidates for preclincal toxicological evaluation (Decision Point IIA) (see Table V). Eight Investigational New Drug Applications (IND) have been submitted to the Food and Drug Administration (see Tables V and VIII). Of the 14942 new crystalline materials added to the program, 606 compounds (4.05%) were acquired under contract; 51 compounds (0.34%) were purchased; 10,250 compounds (68.59%) were voluntarily submitted from industrial sources; 3590 compounds (27.02%) were from non-industrial suppliers. Of the 14942 compounds received; 1615 were confirmed actives which represents 10.92% actives (see Table V).

The Branch manages a total of 20 contracts with industrial organizations, academic and research institutions, both domestic and foreign, totaling approximately 2.6 million dollars and covering the major areas as shown under the primary responsibilities of the Branch. Of the 20 contracts, 15 are domestic and 5 are foreign contracts.

In summary the following were achieved during this report period year by the Drug Synthesis and Chemistry Branch.

1. A total of 22669 new substances (Table V) was obtained for screening or other evaluation;
    a. 14490 synthetic
    b. 1347 antibiotic cultures
    c. 6153 plant extracts
    d. 227 animal extracts
    e. 452 other crystalline materials
2. A total of 606 new compounds were prepared under contract;
3. Sixteen new substances were assigned to Decision Network IIA (Table V) and VII) (candidates for preclinical and toxicology);
    a. 3 fermentation products
    b. 2 plant products
    c. 11 synthetic compounds
4. Eight drugs were assigned IND numbers (Table V and VIII);
5. One thousand six hundred and fifteen compounds were assigned to Step III (Table V);
6. Using preselection technique for compound selection for the screen provided a major increase in the number of actives from 7.2% to 10.92%;

7. Twenty contracts were supervised by the Branch;
   a. 17 synthesis laboratories
   b. 3 miscellaneous
8. There were 5 patent applications reviewed and commented upon;
9. Nine patents issued to our contractors;
10. Samples were received from 159 new industrial concerns and 371 new non-industrial sources;
11. Two hundred and twenty-six manuscripts by the DCT contractors were cleared for publication;
12. Fifty-one papers were published by the Branch contractors and five by staff;
13. Three seminars were sponsored by the Branch.

TABLE V

MATERIAL ACCESSIONED AND ACTIVITY REPORT(1)

	CRUDE EXTRACTS				CRYSTALLINE				DECISION		IND	
	NO. SUBMITTED		NO. ACTIVE		MATERIAL SUBMITTED		STEP III(2)		POINT II(3)		FILED	
	76-7	77-8	76-7	77-8	76-7	77-8	76-7	77-8	76-7	77-8	76-7	77-8
Fermentation Products	2518	1347	92	138	217	205	19	29	4	3	--	1
Animal Products	1537	227	90	52	39	62	--	1	--	--	--	--
Plant Products	8673	6153	567	807	140	185	39	19	1	2	--	2
Synthetics	--	--	--	--	19837	14490	1412	1566	10	11	6	5
	12728	7727	749	997	20233	14942	1470	1615	15	16	6	8

(1) Based on Calendar Year: January 1976-March 1977 (76-7); April 1977-March 1978 (77-8)

(2) Showing Confirmed Activity

(3) Selected Candidates for Preclinical Toxicological Evaluation and Clinical Trial

TABLE VI

SOURCE OF COMPOUNDS

SOURCE	NUMBERS ACCESSIONED	NUMBER OF ACTIVES(*)	PERCENT ACTIVE TOTAL FILE
Non-Industrial	3590	358 (9.97%)	2.43
Industrial	10250	992 (9.69%)	6.71
Government	445	81 (18.02%)	0.54
Contract	606	169 (27.88%)	1.14
Purchase	51	15 (29,40%)	0.10
	14942	1615	10.92

* DN-1 Compounds

TABLE VII

COMPOUNDS PASSING DECISION NETWORK IIA

NSC NO.		
21548	Thymidine	Synthetic
118742	Pentamethylmelamine, hydrochloride	Synthetic
125973	Taxol	Plant
196473	9,10-Anthracenedione, 1,4-bis[[2-[(2-hydroxyethyl) amino]ethyl]amino]-	Synthetic
201628	Carbonic dihydrazide, bis[(3,5-dibromo-2-hydroxyphenyl)methylene]-	Synthetic
237020	Largomycin	Fermentation
234714	Aphidicholin	Fermentation
259272	9H-Purin-6-amine, 9-(5-O-phosphono-β-D-arabinofuranosyl)-, disodium salt	Synthetic
259968	Bouvardin	Plant
261036	1,2-Propanediol, 3-(2-nitro-1H-imidazol-1-yl)- (RO-5-9936)	Synthetic
261037	Misonidazole (RO-7-0582)	Synthetic
282175	Androst-4-ene-3,17-dione, 4-hydroxy-	Synthetic
283162	Trimethyltrimethylomelamine	Synthetic
288411	Indenoquinoline carboxylic acid, sodium salt	Synthetic
296961	Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester) (WR-2721)	Synthetic
526417	Echinomycin (Quinomycin A)	Fermentation

TABLE VIII

IND'S FILED

NSC NO.	COMPOUND NAME	SOURCE
51143	1H-Imidazo[1,2-b]pyrazole, 2,3-dihydro	Synthetic
132319	Indicine, N-oxide	Plant
153353	Alanosine, monosodium salt	Fermentation
165563	Bruceantin	Plant
177023	Levamisole hydrochloride	Synthetic
224131	Pala	Synthetic
249992	m-Amsa	Synthetic
261037	1H-imidazole-1-ethanol, α-(methoxymethyl)-2-nitro-(RO-7-0582)	Synthetic

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DRUG SYNTHESIS SECTION

DRUG SYNTHESIS AND CHEMISTRY BRANCH

SCOPE

The function of the Staff of the Drug Synthesis Section is management of the design and the procurement of unique molecular species as potential antitumor agents. This work is coordinated with all the other facets of the Developmental Therapeutics Program.

The principle activities of the Section are to:

1. Acquire and monitor contracts for the synthesis of compounds destined for evaluation in various biological systems.

2. Plan structure-activity studies and derive new concepts of molecular modification for anticancer drug design.

3. Survey the chemical, biochemical, biological and medical literature to uncover leads to potential antitumor agents.

STAFF

The personnel of the Synthesis Section consists of three professional members and one secretary. The professional staff are project officers for the synthesis contracts and collaborate with the Branch Chief to fulfill the functions of the Section.

SYNTHESIS ACTIVITIES

The objectives of the synthesis section, are to provide the synthesis program with new chemical agents for anticancer evaluation are achieved through synthesis contractors. Their laboratories function to supply compounds of diverse structures and complexity.

Structure-activity studies of known compounds are undertaken to improve their anticancer activity. The parent compound may be a new or novel structure, e.g., a synthetic compound, an intermediate from a reaction sequence, a natural product, an important clinical agent, etc.

New avenues for synthesis may originate from a variety of sources, for example, leads from the screening program, biochemical or biological rationale, and suggestions from staff and consultants. Sometimes compounds are synthesized to resolve the questions on biochemical mechanisms of action.

For convenience, the work of the synthesis contractors for the past year is reviewed in two parts: I - analog development and II - new lead development.

I ANALOG DEVELOPMENT

Nucleosides

The design of fraudulent nucleosides and related heterocyclics as antimetabolites has provided one of the most important sources of anticancer drugs. At least six of these agents are in clinical use today. Present investigations are intended to develop agents which show superior selectivity in the treatment of tumors. The approach utilizes established and developing biochemical rationales to guide the design and synthesis of new agents. The contract with the University of Utah covers the synthesis of a range of nucleosides including bicyclic nucleosides with unusual bases, e.g., deazapurines and pyrazalopyrimidines, C-nucleosides and polycyclic-nucleosides. In addition, the contractor is developing two compounds that have shown promising anticancer activity, a tricyclic nucleoside (NSC-154, 020), and a bicyclic nucleoside containing selenium (NSC-137,679). Both compounds show high antitumor activity and unusual biochemical properties and have passed DN-II.

The Southern Research Institute contract encompasses synthesis of novel purine and pyrimidine nucleosides. Compounds of interest are a variety of nucleosides with the base attached at position other than the normal C-1 position of the sugar, deaminase inhibitors, 2'-deoxyribonucleosides and carbocyclic analogs. 2-fluoro-ara-A was shown to be equivalent to Ara-A plus 2'-deoxycoformycin in anticancer activity against L1210. The fluorine atom prevents deamination but does not effect phosphorylation. An improved method for the preparation of 2-fluoro-ara-A was developed and a patent application was filed. The carbocyclic analog of cytidine was shown to inhibit the conversion of UTP to CTP and appears to inhibit dCTP synthesis.

Two new types of anticancer agents, namely, 2-amino-6-chloro-1-deazapurine and 3,5,8-triaza-7,8-dihydronaphthalene were identified and structure-activity studies were initiated. This contract will be terminated in June.

Anthracyclines

Our objective is to synthesize more effective and less toxic analogs of adriamycin and/or daunorubicin. The synthetic approaches used are focused on the regioselective modifications of either the whole drug or more fundamental changes in each of the constituent parts (sugar and aglycone), followed by coupling to give the final product. The analogs may provide better activity through a variety of actions, for example:

1. Different binding affinity toward different DNA's.
2. Enhanced rate of cell penetration and intracellular distribution.
3. Altered metabolic inactivation.

Work on the intact drugs and the aglycones is contracted to Farmitalia and SRI International (formerly Stanford Research Institute). SRI International has discovered that considerable activity is retained when the aminosugar is replaced by β-alanine in daunomycin.

Farmitalia has produced a modified adriamycin in which the aminosugar has the L-arabino configuration. This compound has been introduced into clinical trials in Europe. They also have synthesized a 4-demethoxy adriamycin analog which possesses excellent activity against P388 and B16 tumors.

The contract at Ohio State University is concerned with the synthesis of aminosugars of differing configuration. Practical synthetic routes to ten daunosamine analogs have been developed and methods for their glycosidic coupling evaluated. Interestingly, a daunomycin analog containing a deaminosugar (the L-lyxo epimer) has shown good activity. This is the first known example of any anthracycline bearing a nonamino sugar which is active. Additional amount of this compound was resynthesized and is currently being evaluated in a panel of test systems. The studies completed permit partial formation of a structure-activity matrix (antitumor activity and toxicity) as an aid to the design of improved drugs. All the anthracycline contracts will be phased out in June.

Through the synthesis and structure-activity study of a series of 1,4-bis (substituted alkylamino)quinones, Midwest Research Institute designed and prepared the highly active 1,4-bis(hydroxyethylamino)-6,8-dihydroxyanthraquinone. This compound is active against the adriamycin and daunomycin resistant tumor lines and is presently undergoing extensive evaluations in advanced screening, mechanism of action and cardiotoxicological studies as a candidate for clinical trials. A series of second generation analogs were also made.

Alkaloids

Work at the University of British Columbia to synthesize analogs of Vincristine and vinblastine is proceeding quite well. A series of investigations have given valuable insights into the chemistry of the indole unit of the bisindole alkaloids. A group of novel dihydroindole derivatives synthesized in earlier work by the contractor have been coupled to catharanthine to form novel dimers. Various significant improvements have been made in the area of selective functionalization of 3',4'-dehydrovinblastine. These provided efficient overall yields of leurosine, N-formylleurosine (a clinical agent) and other bisindole derivatives. Most significantly a general method has been recently developed for the controlled oxidative conversion of vinblastine (N-methyl) derivatives to the corresponding Vincristine (N-formyl) compounds. In general, many of the compounds produced under this program have shown good antitumor activity and reduced toxicity in comparisons with the natural drugs. One of the synthetic alkaloids (NSC-250-833) is undergoing further study at the Cancer Control Agency of British Columbia in Vancouver for possible use in the treatment of human cancer.

Biosynthetic investigations of the cell free extracts from *Catharenthus roserus* plants have shown that vindoline can be produced from simple molecules. This observation suggested the study of crude enzyme preparations as a potential method for producing the clinically important dimeric indole alkaloids such as Vinblastine.

Midwest Research Institute reports that the alkaloids Coralyne, nitidine and related alkoxyphenanthridinium salts inhibit the enzymes O-methyltransferase and t-RNA methyltransferases found in malignant tumor tissues. None of these alkaloids or their analogs demonstrated mutagenicity in the Ames test, suggesting that the presence of a quarternary nitrogen atom and alkoxy groups could be important in alleviating the mutagenicity of mutagenic and carcinogenic hydrocarbons.

Folic Acid

The objectives for the synthesis of analogs of folic acid are to obtain agents: a) with more favorable transport properties, b) and to synthesize compounds that inhibit enzymatic systems other than dihydroreductase.

Southern Research Institute has made a series of α-amides of the antitumor folate, methotrexate. Substitution at the α-carboxyl of methotrexate has been shown not to interfere with binding to dihydrofolic reductase or to thymidylate synthetase but has a profound effect on the transport of the analogs into cells. The studies could lead to an improved methotrexate derivative if selective transport into tumor cells can be achieved. The synthesis of analogs of methotrexate with modification at the $\underline{N}^{10}$ position have been undertaken in an effort to obtain compounds with altered binding properties. Midwest Research Institute has completed the synthesis of an analog bearing a CH_2 group at position-10 instead of a CH_3-NH and a quinazoline moiety in place of the pterin structure on C-9.

Cyclosphosphamide

It is now well documented that cyclophosphamide, a clinically effective antineoplastic agent, is activated in the liver by an oxidative process. Although the principle oxidative metabolic pathway has been well defined, the exact nature of the metabolites responsible for the chemotherapeutic activity and selectivity has not been fully clarified. The work at Collaborative Research, Inc. is directed toward clarifying the roles of aldophosphamide, 4-hydroxycyclophosphamide and iminophosphamide, all of which are intermediates in cyclophosphamide metabolism. In this area, the contractor has synthesized and separated the diastereoisomes of 4-cyanocyclophosphamide. Significant progress has been made on the synthesis of ten additional compounds.

Metal Complexes

Transition element coordination compounds continue to offer unusual opportunities for development of effective antitumor agents. The contractor has synthesized 38 new complexes, of which 13 (40%) are active against murine leukemia. It has been established that antitumor activity increases with increasing ring size of the 1,2-diaminoalicyclic ligand in a series of platinum malonato, phthalato, and tartrato complexes. Work also has shown that bidentate chelate platinum complexes with nitrogen donor groups give consistently superior activity over that of similar complexes with other transition elements. A selanato complex showed excellent activity; other platinum complexes containing germanium, arsenic, and antimony are on test.

A significant development is the synthesis of the water soluble platinum compound, NSC-271674. It appears to have less renal toxicity than diammino dichloroplatinum. Larger quantities of this compound were synthesized to meet the demands for further studies.

Central Nervous System Agents

CNS-penetrating antineoplastic agents are an essential part of the treatment of primary CNS neoplasms and lesions metastatic to the brain. The difficulty in eradicating tumor cells in the CNS and especially in the brain is due, in part, to the inability of drugs to cross the blood-brain barrier. The development of CNS-penetrating antitumor agents has involved the synthesis of lipid soluble compounds with antineoplastic activity. Northeastern University has synthesized two classes of compounds, aporphine mustards and aminoisoquinoline mustards, as potential CNS antineoplastic agents. Approximately 60 compounds have been synthesized and tested in the L1210 and PS systems. Several aporphine mustards have shown promising activity. PS data for two of these indicate significant activity. One has also been tested in B16 and has a T/C of 170 at 50 mg/kg in this system. This aporphine mustard (NSC-279847) is scheduled for some of the intracerebral tumor systems.

Radiosensitizers

This area of research is devoted to the development of chemical agents which will selectively sensitize hypoxic tumor cells, thereby increase radiation response, and enhance tumor destruction. The design of chemical radiosensitizers is directed toward those that mimic the oxygen effect, show activity against hypoxic cells only, are non-toxic at radiosensitizing concentrations, work effectively against proliferating and non-proliferating cells, diffuse rapidly into the hypoxic areas of tumors and are slowly metabolized. Several classes of compounds are reported as having radiosensitizing effects, such as, nitroimidazoles, thio-sugars and dihalopyrimidines. The nitroimidazoles are currently among the more promising agents. Two contractors are assigned to do research in this area: the Institute of Cancer Research, Royal Cancer Hospital and SRI International. Both of these contractors are synthesizing nitroimidazoles with electron-affinic characteristics and evaluating their radiosensitizing and cytotoxic properties. The Institute of Cancer Research is synthesizing 5 and 1-substituted 2-nitroimidazoles. The work being done at SRI International is concerned primarily with 1-substituted 2-nitroimidazoles. These two contracts have been initiated recently. Physical chemical measurements as well as *in vivo* and *in vitro* testing are also a part of these contracts.

Natural Product Fragments

Bleomycin is an antibiotic and effective antitumor agent with clinical activity reported against lung cancer and certain lymphomas. Bleomycin is a very complex molecule with 19 asymetric centers. To facilitate synthesis the molecule was divided into five unique fragments, with each division at an amide linkage. The individual fragments were biologically evaluated to determine possible sites of anticancer activity. The work at Massachusetts Institute of Technology is concerned with the synthesis of analogs of these fragments and their combinations. The contractor has synthesized and submitted for biological evaluation 22 new compounds. Each of these compounds was either an analog of the five active species or structurally related to one of the Bleomycin fragments. Five intermediates prepared as Bleomycin fragments were found to have some antitumor activity.

II NEW LEAD DEVELOPMENT

In many tumors the salvage mechanism is the predominant source of pyrimidines for the synthesis of the DNA and RNA; the natural macromolecules used in cellular function and reproduction. St. Jude Children's Research Hospital is engaged in the synthesis of two main classes of enzyme inhibitors for the pyrimidine salvage pathway. These are the nucleoside analogs of 1- and 2-deazapyrazofurin and 1,2,4-thiadiazole. The sixteen step synthesis of the pyrazofurin analogs, although tedious, is proceeding satisfactorily. A considerable quantity of 5-ethoxy-2-(1-β-D-ribofuranosyl)-1,2,4-thiadiazoline-3-one has been synthesized for conversion to the desired 3,5-dione and the 5-amino-3-one analogs. This contract will be phased out in early 1979.

The contractor at the University of Southern California is engaged in the synthesis of two unique types of potential antitumor compounds. One of them is a derivative of a cyclopentanoisoquinoline which is postulated to a simple model of a DNA intercalating agent. A N-carboethoxy cyclopentanoisoquinoline has been successfully synthesized and it shows activity against cultured lymphoblastoid and L1210 cells. Preparation of larger quantities of the base is now underway for the synthesis of the corresponding N-ribosyl derivative. The objective is to synthesize compounds with enhanced intercalation properties.

Significant progress has been made in the synthesis of the potential "suicide inhibitor" 5-ethynylorotic acid. Two synthesis approaches are under study and both have given the necessary intermediates for the elaboration of the desired product.

Our PL-480 contract agreement with Central Drug Research Institute provides us a variety of compounds in sufficient quantities for anticancer evaluation. These materials arise from several sources, (a) synthesis of aromatic compounds containing a variety of functionalities such as nitro, isothiocyano, etc., (b) synthesis of nitrogen and nitrogen-sulfur heterocyclics, (c) the modification of natural products with anticancer activity, for example, alkaloids, antibiotics, peptides, etc., (d) collection extraction, and isolation of Indian Medicinal plants. In addition, selected compounds from their internal programs in other disease areas are made available to us for anticancer evaluation. Six structurally modified ellipticines, two emetine analogs, several nitrogen-containing heterocyclics, aromatic isothiocyanates, and aromatic nitro compounds were synthesized. Four compounds have shown activity in P388 tumor system.

TABLE IX

CONTRACT LABORATORIES FOR SYNTHESIS

Contractor	Investigator	Contract No.
British Columbia, Univ. of	Kutney	N01-CM-23223
Central Drug Research Institute	Dhar	PL-4-8001
Collaborative Research, Inc.	Friedman	N01-CM-77071
Farmitalia	Bernardi	N01-CM-57036*
Farmitalia	Arcamone	N01-CM-57014*
Institute of Cancer Research	Adams	N01-CM-77139
Massachusetts Institute of Technology	Hecht	N01-CM-43712*
Midwest Research Institute	Cheng	N01-CM-33743*
Northeastern University	Neumeyer	N01-CM-53741*
Ohio State University	Horton	N01-CM-57015*
St. Jude Children's Research Hospital	Welch	N01-CM-77127
Southern California, University of	Heidelberger	N01-CM-67084
Southern Research Institute	Montgomery	N01-CM-43762*
Stanford Research International	Acton	N01-CM-33742*
Stanford Research International	Lee	N01-CM-87207
Utah, University of	Townsend	N01-CM-77142
Veterans Administration Hospital	Gale	Y01-CM-60102

*Contract terminated during this report period

CHEMICAL AND DRUG INFORMATION SECTION

DRUG SYNTHESIS AND CHEMISTRY BRANCH

Scope

The Chemical and Drug Information Section is responsible for: (1) operating, maintaining, and enhancing the automated Chemical Information System (CIS) which supports the accessioning, storage, and distribution of chemicals and drugs acquired by the Developmental Therapeutics Program for antitumor testing, including the coordination of staff and contract activities related to the CIS; (2) managing the storage and retrieval of chemical information in both automated and manual files; (3) conducting structure/activity studies to optimize acquisitions by identifying chemical classes to be deemphasized because they are largely negative or adequately studied and by developing models for determining the probability of antitumor activity in the prescreen for potential acquisitions; and (4) studying various methods for identifying compounds of interest for acquisition which are reported in the literature or are in various chemical files maintained by governmental agencies, industrial organizations, and information venders.

Staff

The Section's staff consists of two chemists, two system analysts, one mathemetician, one chemical information clerk, and two supporting clerks.

Emphasis of Effort

During this report period, the Section concentrated on: (1) operating the CIS and implementing various changes to enhance its performance; (2) providing chemical information to the Drug Synthesis and Chemistry Branch (DS&CB) and other DTP decision-makers; (3) improving and installing in an experimental mode an automated, statistical model which correlates chemical fragments of previous acquisitions with their biological activity for use in optimizing the selection of acquisitions for antitumor screening; (4) generating structural diagrams for compounds registered before the start of the CIS to better meet informational requirements for substructure search output and for other purposes; and (5) continuing the experimental surveillance of the literature to identify compounds which warrant acquisition based on structural characteristics or specific biological properties.

Chemical Information System (CIS)

This automated system enables a limited in-house staff to monitor the accessioning, storage, and distribution of chemicals and drugs acquired for antitumor testing. In processing acquisitions, the system identifies duplicates and analogs of Program interest, produces action and informational documents required by the staff and contractors involved with accessions, maintains inventory control, and tracks acquisitions from their receipt through shipment. In addition to accelerating the processing of accessions, the CIS provides improved operational control in monitoring the contractors engaged in this activity. In April, 1978, the system stored and permitted searching information on 316,000 compounds. An index of names is generated and maintained to determine whether a named compound is in the DS&CB collection or has been reported in Chemical Abstracts thereby facilitating access to the literature.

In response to Program requirements, a wide variety of enhancements were made to the CIS, including: (1) increasing the efficiency of the automated method by which the structures of all new acquisitions are examined to determine which are in the 32 chemical classes for which special testing is required; (2) modifying the information in a machine-processable form which is transferred to the Biological Data Processing System (BDPS) as a result of that system's redesign; (3) displaying additional information on hard copy reports and on the terminals used for retrieval, including such information as the reason for acquisition or the reason for the manner in which the compound was tested, and transferring these and related data to the BDPS; (4) establishing automated procedures to minimize the acceptance of compounds having structures identical to previous acquisitions; and (5) improving the timeliness and reliability of processing recurring, highly complex correction transactions (e.g., when a supplier corrects the structure of a previously-donated compound) by increased automation to assure the corrections of all related files without substantial manual intervention by a chemist.

Since the startup of the CIS in July, 1974, the structural images of 81,000 compounds have been captured by registration and are displayable on hard copy and on the terminal used to query the data base. However, structural images were not available for the 225,000 compounds registered by the previous chemical information system. Structural images for 184,000 were generated during this period and added to the file. These images were created by using a program developed by CAS for its own use called Algorithmic Structure Display which creates a structure image from the machine record describing the atoms in the molecule and their bonds. Thus, images can now be retrieved for 86.6% of the 316,000 compounds on the file as of April, 1978. Of the 41,000 compounds registered by the CIS but without structure images, approximately 8,000 structures will be generated by another program under development and the 32,000 remaining structures will require input by OLSIS (another CAS-developed program) which enables the capture of complex structures, including stereochemical information, by the use of a light pen on a cathode ray tube.

Automated Retrieval of Information

The on-line INQUIRY subsystem of the CIS was heavily utilized during the past year to support the information needs of a wide range of organizational units within DCT. The data base of the system, available to DTP staff and contractors involved with the acquisition activities, is maintained under contract at CAS in Columbus, Ohio and is accessed from Washington using alphanumeric and graphic terminals.

INQUIRY is used to search the file of 316,000 chemicals tested since 1956 for the presence of individual compounds or their corresponding salts or stereoisomers and for compounds containing a common substructure. Nonstructural data, such as who supplied a given chemical and how much is still available in inventory, can also be retrieved. INQUIRY is used routinely to compare to prior acquisitions all new submissions which are to be sent for priority testing as well as to determine the novelty of new active structures which are reviewed by the Data Review Subcommittee and the Drug Evaluation and the Decision Network Committees. This review for novelty is a key factor in the scheduling of tumor panel testing and the further development of a given drug. The system is also used in the evaluation of our prior experience in a class before new synthesis programs are begun or structure-activity studies are started. The Section answered more than 500 requests for information, including more than 100 requests for searching individual compounds based on structure (full structure search), 350 requests for the compounds in specific chemical classes (substructure searches), and the balance of the requests for nonstructural information. See Table X for a listing of representative substructure searches.

TABLE X

SOME SUBSTRUCTURE SEARCHES OF INTEREST

Description of Search	Number of Compounds
Antifols	
Methotrexate derivatives	336
Triazine antifols (Baker)	844
Bis acylating agents	295
Ellipticines	113
"Fraudulent" nucleosides	121
Nitrosoureas	386
PALA	186
Platinum	814
Sesquiterpene lactones	162
Survey of foreign supplier	
India	2,460
Soviet Union	106

Deletion of 50 known active antitumor classes from the training set of the preselection model to improve identification of novel actives.

Review of all metals, including total collection and Selected Agent Compounds (SAC).

Subdivision of all SAC nucleosides in subclasses for review by Data Review Subcommittee.

Survey of bioisosteric properties of a wide variety of functional groups.

Various searches by investigators outside the Program requested under the Freedom of Information Act.

INQUIRY was significantly improved by several programming enhancements. A change in the method of accessing structures for atom-by-atom searching resulted in nearly an order of magnitude improvement in terminal response time for multi-structure queries with a lesser but welcome improvement in the cost of such queries. In addition, a new capability to save the results of searches and use them as keys for further searching of the information retrieved also proved useful and cost-effective during the year. With the addition to the data base of structural images for compounds acquired prior to the startup of the CIS, increasing use was made of the graphics capabilities of the Tektronix terminal to display structures on-line. During the coming year, this augmented capability will allow the phasing down of the current manual photocopying of structures for compounds retrieved from searches. More importantly, it will provide a more effective means of browsing during a substructure search and encourage the use of INQUIRY by investigators in DCT areas other than this Section. The Section also maintained expertise in the on-line systems offered by the National Library of Medicine (NLM) such as Chemline, Toxline, and Cancerline and used these as additional aids in the search for requested information. The Section participated in an evaluation of proposed extensions to NLM's Chemline substructure search capability which will be used to plan improvements to this system.

Structure/Activity Studies

The analysis of the chemical and biological data bases was continued to develop improved acquisition guidelines and automated mechanisms for optimizing the selection of compounds for antitumor testing. As significant milestones are reached, results are presented to the Preselection Committee which was established to advise the DS&CB on improving methods to optimize acquisitions.

Adequately Studied Classes. Chemical classes heavily represented in the collection were queried and analyzed to specify the total number of compounds and the number of actives in each class and subclass Tables XI and XII report for purines and pyrimidines the number of compounds in the DTP collection with a given substituent pattern of either ring and the number of such compounds which have demonstrated antitumor activity. Based on this study, the following purine subclasses may be considered adequately studied: hypoxanthines; caffeines; and 2-amino, 6-oxy (guanine). Among the pyrimidines, the following subclasses may be considered adequately studied: 2, 4, 6-trioxy (Barbituric Acid); 2, 4, amino, 6 (oxy or thio); 2 amino, 4 (oxy or thio), 6 (not N); and 4 (oxy or thio), 2 (not N, O, P, S).

TABLE XI

ANALYSIS OF PURINE SUBCLASSES

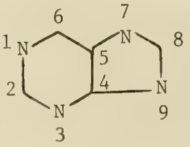
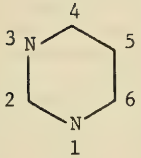
Title of Class		Total File	All Actives	Significant Actives (A)	Normalized Activity Ratio (B)
Purine	 1 2 3 4 5 6 7 8 9	4796 (C) (1191) (D)	339 (103)	195 (51)	2.8 (3.4)
1) 6-alkyl		75 (8)	4 (2)	0 (0)	2.1 (0)
2) 6-halo		230 (66)	14 (6)	10 (4)	2.4 (3.6)
3) 6-oxy (hypoxanthine)		413 (88)	4 (1)	3 (1)	.4 (.5)
4) 2, 6-oxy (caffeine)		885 (9)	4 (0)	2 (0)	.2 (0)
5) 2, 6, 8-oxy (uric acid)		59 (1)	0 (0)	0 (0)	0.0 (0)
6) 2-amino, 6-oxy (guanine)		230 (80)	1 (1)	0 (0)	.2 (0)
7) 6-mercapto		807 (158)	146 (26)	103 (18)	7.2 (6.6)
8) 2-amino, 6-mercapto (thioguanine)		302 (98)	93 (33)	54 (16)	12.3 (13.5)
9) 6-amino (adenine)		1558 (679)	51 (21)	15 (3)	1.3 (1.2)
10) 6-seleno		20 (17)	7 (5)	3 (3)	14.0 (11.8)

TABLE XII

ANALYSIS OF PYRIMIDINE SUBCLASSES

Title of Class		Total File	All Actives	Significant Actives (A)	Normalized Activity Ratio (B)
Pyrimidine		10,000(C)	290	94	1.2
1) 2, 4-dioxy (uracil)		2,222	74	26	1.3
1a) -5 carbon subst, (Thymine)		787	18	2	.9
1b) -5 fluoro (5-FU)		101	19	9	8.0
1c) -5 Cl Br. I (5-IUDR)		105	3	3	1.1
1d) -6 acyl (Orotic Acid)		71	5	3	2.8
2) 2-oxy, 4-amino (cytosine)		811	60	22	3.0
3) 2,4, 6-trioxy (Barbituric Acid)		1,450	7	1	.2
4) 2,4, 6 triamino		240	9	2	1.5
5) 2,4, amino, 6 (oxy or thio)		256	1	0	.2
6) 2,4, amino, 6 (not N,O,P,S)		771	32	7	1.7
7) 4 amino, 2, 6 (not N,O,P,S)		396	7	0	.7
8) 2 amino, 4 (oxy or thio), 6 (not N)		777	7	0	.4
9) 2 amino, 4, 6 (not N,O,P,S)		683	15	8	.9
10) 4 (oxy or thio) 2 (not N,O,P,S)		400(E)	3	1	.3
11) 2 (oxy or thio) 4, 6 (not N,O,P,S)		359	7	2	.8
12) 2, 4, 6 (C or H only)		850	34(F)	26(G)	1.6
13) 2, 4, 6 (not N,O,P,S,C)		180(E)	3	0	1.1
14) 4, 6 amino, 2 (not N,O,P,S)		165	2	0	.5

NOTES FOR TABLES XI and XII

(A) Compounds with reproducible activity in a DTP test system which are in Decision Network 2 or above.

(B) Normalized activity ratio equals:

$$\frac{\text{actives in a given class/all compounds in the collection that are in that class}}{\text{all actives in the collection/all compounds in the collection}}$$

(C) The Chemical Abstracts Service has reported on 27,000 purines and 57,000 pyrimidines.

(D) Numbers in parenthesis indicate the total number of corresponding nucleosides.

(E) Estimated

(F) Includes 26 Terephthalanilide analogs

(G) Includes 25 Terephthalanilide analogs

Probability-based Preselection Model. Significant progress has been made in developing a model to predict the probability of antitumor activity of potential acquisitions in the lymphocytic leukemia (P388) prescreen. This model is based on statistics regarding structure-activity relation over varied classes of structures which are secured from the data bases of the chemical and biological data processing systems. The estimate of the probability of activity determined by the model is brought to the attention of medicinal chemists selecting compounds for acquisitions. In addition, there is provided a measure of rarity or uniqueness, i.e., the occurence of structural fragments infrequently or not present in the compounds previously collected. The Notice of Intramural Research Project (Number Z01-CM-07 101-02 DSCB) which follows further describes the performance of the model.

The analysis of results from testing an earlier model upon approximately 4,500 compounds demonstrated that the acquisition of high scoring compounds would usefully enrich the proportion of actives. Further tests demonstrated that when the model was constructed from the training set which excluded compounds in chemical classes which are known to display antitumor activity the performance of the model improved with respect to assigning high activity scores to novel active compounds. It should be noted that another processor (called Special Structure Identification) will identify which potential acquisition are analogs of well-known active agents.

The model has been installed at the Chemical Abstracts Service and will be used in an operational mode albeit experimentally to test the utility of using the model on new potential acquisitions.

Literature Surveillance and Other Selective Acquisition Experiments. The investigation of methodologies for selective acquisition was continued, using approaches stemming from chemical characteristics or biological properties of Program interest. Some experiments were initiated by questions raised by an *ad hoc* DTP committee investigating the feasibility of mounting a literature surveillance effort. Other experiments were initiated to determine the utility of emerging automated capabilities in literature surveillance. The results of several experiments are reported below.

Substructure searches for analogs of PALA (NSC 224131) and 2-nitroimidazoles (NSC 105831) were made with the cooperation of the Basel Information Center for Chemistry (BASIC) against the *Chemical Abstracts* data base of 4,000,000 compounds. Fifty-nine compounds in 23 articles were retrieved for the PALA search. The BASIC search provided the *CA* abstract numbers of pertinent articles and the CAS Registry Numbers assigned to each of the compounds reported in each article. With this information, CAS then used their automated methods to: (1) output the structural diagrams of the retrieved compound by the Algorithmic Structure Display program; and (2) input the structures into the CAS Registry System to report by the Search on Topology routine which of these were duplicates or near-duplicates of compounds in the DTP collection. Copies of the abstract or papers, as required, were provided. Of the 59 PALA analogs, only four were duplicates or near-duplicates of DTP compounds. This information was furnished to the Acquisition Contractor to solicit samples. An analysis of the 86 abstracts and 306 compounds output by the search for 2-nitroimidizoles has not yet been completed.

Other literature searches were based on biological properties of Program interest. A trial search of current input to the *Chemical Abstracts* data base for articles reporting compounds which inhibit the production of macromoleculaes (primarily DNA, RNA, and protein) demonstrated the utility of using this search strategy for the selective acquisition of compounds with a high probability of antitumor activity. A search profile was developed for searching CASIA, (Chemical Abstracts Subject Index Alert) which is a computer-readable index to documents reported by Chemical Abstracts. In a manner similar to that described above, CAS used their resources to provide information on the data retrieved. An analysis of the search results for a two week period indicate that 20 of the 31 articles retrieved were relevant and these articles described 35 compounds of which 23 were in the DTP collection. The reported biological properties of the remaining 12 compounds warranted the action taken for acquisition. It is significant to note that of the 23 reported compounds that were in the DTP program many displayed significant antitumor properties, i.e., the drug was in general practice, had proven to be effective in man, or its IND had been withdrawn due to toxicity in man.

An analysis of all compounds determined by the U.S. Department of Agriculture (USDA) to be chemosterilants demonstrated that 34% of such compounds present in the DTP collection displayed some degree of antitumor activity. See Table XIII below. Aside from soliciting chemosterilants in chemical classes which correlated with antitumor activity, these data provided a source of compounds for which an additional supply was needed to complete previous testing.

TABLE XIII

ANALYSIS OF USDA CHEMOSTERILANTS

CLASS	USDA ACTIVES	NOT IN NCI COLLECTION	IN NCI COLLECTION		
			TOTAL	ACTIVE	INACTIVE
Alkaloids	3	0	3	2	1
Aziridine	60	31	29	18	11
Boron compounds	8	8	0	0	0
Dithiazoliums	9	8	1	1	0
Epoxides	2	2	0	0	0
Metal complexes	4	2	2	1	1
Methane sulfonates	7	2	5	2	3
Nitrogen mustards	10	3	7	4	3
Phosphoamines	25	18	7	0	7
Triazines	45	27	18	0	18
Sulfonamides	15	14	1	0	1
Ureas	42	37	5	1	4
Miscellaneous	34	16	18	4	14
TOTAL	264	168	96	33	63

Plans for the Chemical Information System in the Coming Year. Structure Output. With 85% of the structure images on the file and available as on-line display and hard copy output from substructure search and reports by the CIS, it would be advantageous to input the remaining 32,000 structures which could not be generated algorithmically. However, these complex structures require an expensive input method, including terminals with light pens supported by on-line, interactive input programs at CAS. Because of funding constraints, input cost will be spread over three years. Adding the structure images onto the CIS files is another step towards effectively interlinking the chemical and biological data bases for combined reports.

The Statistical Preselection Model. The existing model will be tested in a pilot operation to determine the extent to which it will aid a medicinal chemist to optimize the selection of compounds for acquisition. The model will output for each potential acquisition a report of its activity score and its unique or rare key, i.e., a chemical fragment present in the potential acquisition which is not or infrequently present in prior acquisitions which had been tested in the P388 lymphocytic leukemia test system. Also available to the decision-maker will be the output of Search on Topology. This program reports which potential acquisitions are duplicates and near-duplicates of compounds in the DTP collection and for each such duplicate additional information such as the status of active compounds in the linear array.

Concurrently, additional or alternative features (structural components) will be devised and tested to overcome problems of context and redundancy in the present model. Information currently available in the CIS, (e.g., GAA keys, ring identifier numbers, etc.) will be analyzed to select potentially useful features and data generated for incorporation in the model for trial purposes. Linear sequence keys, TSS keys, and other large structural fragments which provide more definitive topological information will also be investigated. Features which improve the prediction of activity or better identify unique or rare keys will be incorporated into the model.

Design a Preselection Subsystem: A design will be made of a Preselection Subsystem to aid making the optimal selection of compounds to enter the prescreen. The Preselection Subsystem will integrate the two components described above (i.e., the preselection model and the Search on Topology processor) with the Structural Feature Identification (SSF ID) processor which will be optimized. SSF ID identifies compounds in chemical classes which are: similar to those being synthesized under contract; to be deemphasized because they are largely negative or adequately studied; or to be emphasized because they are in active classes not adequately represented in our collection. A report which consolidates information regarding a given potential acquisition from all three processors will facilitate decision-making. The pilot operation of the 3PS preselection model for the first six months of the contract will permit us to develop a realistic design.

INQUIRY. Additional search keys will be created to permit making queries now impossible or now made very awkwardly and expensively but which are required to meet operational demands for information. For example, the availability of a key for the Confidentiality Code will permit limiting queries from investigators outside the Program to non-discreet compounds and avoid the generation of unused Chemistry Cards and biological data for discreet compounds. The QNS key (replenishment action) will facilitate reports in support of efforts to follow up on requests for additional material made to suppliers. The key for the Criteria Code will support the Acquisition Contractor in making retrospective studies on acquisitions possessing structural characteristics which are believed to correlate with antitumor activity.

Another planned improvement in INQUIRY will permit a user to specify a search qualification expression in Boolean logic to qualify the search answers. Selected data elements on the file will than be examined for such conditions as =, <, >, within ranges, etc. An example of the use of this capability would be to identify all nucleosides by a substructure search and to reduce the answers by qualification to those with a supply of more than 0.5 grams.

Other INQUIRY enhancements will be programmed, including: the display on the terminal of the connection table for a structural query to facilitate its modification based on the review of preliminary results; the ability to specify a partial structure when searching for the presence of possible duplicates in a large number of congeners; and the ability to limit the search to a range of NSC numbers to avoid searching the entire file.

Inventory Subsystem Study. The Inventory/Shipping processor will be modified to accept and process inventory data for compounds acquired before the start of the CIS (Phase II), i.e., all compounds with NSC's below 265500. Rather than the remaining weights of individual samples received for a given NSC, data on the inventory card reporting the total balance on hand will be processed. The inventory of the backfile will be input to the extent resources are available to the Storage and Distribution Contractor. When all of these data are input to the CIS, the manual inventory control card system will be retired and the automated system will be used exclusively. The availability of inventory information for all compounds in the automated system will speed the work of DEB, DS&CB, and Microbiological Associates, Inc.

Miscellaneous Programming Changes. In addition to the major developmental tasks described above, many non-major modifications to the CIS are required in response to changing operational requirements. Typical of such modifications are the following: (a) modification of the existing Special Structural Feature (SSF ID) processor to generate on a one-time basis SSF codes for all SAC compounds and the transfer of this information to the DEB's Biological Data Processing System (BDPS) to classify structurally all active compounds (and to perform these tasks biweekly for newly-designated SAC's); and (b) the capability of outputting chemistry cards (including NSC numbers and structures) in various formats in response to a chemical query as well as generating and transferring machine-processable records of NSC numbers for input to the BDPS for securing the correspondence biological data. These changes will facilitate interlinking the chemical and biological data bases.

Hardware and Communication. With the ability now to display 85% of the structures in the file (and 100% within three years) and to overcome maintenance problems encountered with the terminals now in use, other available terminals will be evaluated to identify more reliable replacements with the capability of displaying structures efficiently. The search subsystem (INQUIRY) will be modified to support this new terminal. Also, the programs which support the network of terminals linked to the CIS will be modified to increase the availability of terminal hours to the Storage and Distribution Contractor to facilitate the input of inventory data for NSC's below 265500.

TABLE XIV

CONTRACTS MONITORED BY THE SECTION

Contractor	Principal Investigator	Contract No.
Chemical Abstracts Service	Dunn	N01-CM-43722

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM-07101-03 DSCB

PERIOD COVERED
October 1, 19 77 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Computer Methods for Drug Preselection Based on Structure-Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Hodes	Research Mathematician	DS&CB, NCI
OTHER:	G. Hazard	Chemist	DS&CB, NCI
	S. Richman	Head, Chem. & Drug Info. Sect.	DS&CB, NCI
	R. Geran	Biologist	DEB, NCI

COOPERATING UNITS (if any)
Chemical Abstracts Service
Starks, C. P.

LAB/BRANCH
Drug Synthesis and Chemistry Branch

SECTION
Chemical and Drug Information Section

INSTITUTE AND LOCATION
NCI, NIH, Silver Spring, Maryland 20910

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was initiated to provide information about chemical structures as an aid in selecting from potential acquisitions, compounds for screening. Statistics on structure-activity relations over broad classes of structures are being used to predict the probability of antitumor activity of new compounds in the mouse lymphocytic leukemka (P388) screen. In an attempt to uncover novel classes of active compounds, known classes of analogs have been removed from the training set. Also, a measure of rarity or uniqueness of a new structure is derived from the statistics on the structural characteristics of compounds that have been adequately screened in P388, regardless of their activity. The P388 model has been integrated into the DTP Chemical Information System for use in an experimental mode to determine whether it will aid in the selection of compounds for screening. This model will be updated as experiments using new chemical features demonstrate an improvement of performance.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION

Objectives:

1. Evaluate the potential of structure-activity determination over ranges of compounds for specific biological test systems.

2. Demonstrate the feasibility of such methods to preselect compounds for antitumor screening, e.g., in setting priorities or in selecting appropriate test systems for screening.

3. Within the screening capacity, maximize the acquisition of additional promising compounds or compounds inadequately represented in the file.

4. Install these methods into the Chemical Information System operating environment.

Methods Employed

1. The method for predicting the probability of activity uses known biologically active and inactive compounds as training sets in such a manner that the incidence of molecular fragment keys in these sets allows for estimating a measure of the probability of activity of new compounds containing some of these fragments. The original method described in reference (1) uses the incidence of fragments in the DTP collection as a standard. A new version of the method computes estimates from the training sets without requiring a standard. This modification is important for the model when analogs are removed from the training sets without the necessity to remove them from the entire collection.

2. Molecular structure fragments that were previously developed as keys for querying the file of the automated DTP Chemical Information System are being employed as predictor variables. Other sets of fragments are being investigated.

3. Biological data, now limited to the P388 screen, is obtained from a query of the DTP Biology File. The criterion for activity is a confirmed increase in life-span (ILS) of 20%. Active compounds with a confirmed increase life-span of 75% or more are kept separately. Also obtained was a set of negative compounds i.e., those which were tested adequately and failed to increase the life-span by 20% or more. The original training set was limited to compounds below NSC 260000. A new search of the Biology File has been performed to update the model with data on compounds through NSC 300000.

4. Two test sets were processed through the earlier model. These compounds were acquired subsequent to compounds in the training set. Test set 1 consists of 2,332 compounds from NSC 260001 to 265000 with adequate biological and chemical data. Test set 2 contains 3,263 compounds from NSC 268000 to 272000.

5. Analogs of well-known active compounds were then removed from the original training set. This reduced compounds in the training set with a confirmed increase in life-span: of 75% (hereinafter called A's) from 879 to 106: and of 20% but less than 75% (hereinafter called C) from 1,916 to 1,182. Negatives (N's) were reduced from 15,524 to 14,357. The objective was to develop a model which would help identify new active classes. Since the C's were now more than 10 times as numerous as the A's, a (5A + C) model was used in which the chemical fragments in the A's were repeated 5 times as opposed to the earlier (2A + C) model. The two test sets in paragraph 4 above were run through this new (5A + C - analogs) model.

6. To compare the relative effectiveness of the (2A + C) model and the (5A + C - analogs) model in ranking potential accessions, the second training set (resulting from the removal of well-known analogs from the initial training set) was run through both models. An analysis was made of the results using two scales of activity scores for determining the decile in which the activity score of a candidate compound fell. One scale was the range of activity scores achieved by running test set number 1 of paragraph 4 above through each model. The other scale was the range of activity scores similarly derived for test set 2.

7. To get a measure of the rarity or uniqueness of new compounds, a list was compiled of all the keys appearing in P388 tested compounds and their incidence. When a potential acquisition is being examined by the model, its least frequent key is printed together with its incidence. If the compound has a key which did not occur on the list, this unique key is reported.

Major Findings

1. A comparison of the results of evaluating using both test sets with the earlier model may be seen in Table XV. The results for the later test set with more varied structure were not as spectacular as the earlier test set, but substantial enrichment of active compounds among the top 50% still occurred, i.e., were acquisitions limited to compounds whose activity scores fell in the upper half. The percent of active compounds with activity scores in the upper half was 7.9% compared with original 5.4% for the entire test set.

2. A more complete standard for expected performance of a statistical model of this type is its performance on the training set itself. Using the 50% cutoff given by test set 1, there were 24 out of 834 A's and 514 out of 1,776 C's in the lower half or 538 out of all 2,601 actives were in the lower half. This result provides an enrichment factor of 1.59 (i.e., 79.3%/50%) as compared to factors of 1.85 and 1.49 in tests 1 and 2 respectively.

3. The original test sets were processed through the (5A + C - analogs) model. Performance on the novel actives was improved slightly but these results are inconclusive because of the small number of novel active compounds. Again the large range of novel actives in the training set provides a better indicator.

TABLE XV

SEPARATION OF ACTIVE COMPOUNDS BY THE P388 LYMPHOCYTIC LEUKEMIA PRESELECTION MODEL

ACTIVES IN TEST SET 1

111 compounds with confirmed T/C ≥ 125, including
26 compounds with T/C ≥ 175

RANK (Deciles)	NUMBER OF ACTIVES	
	T/C ≥ 175	T/C < 175 ≥ 120
1 (lowest)	0	0
2	0	2
3	0	2
4	1	1
5	0	2
6	0	4
7	2	5
8	0	8
9	8	20
10 (highest)	15	41
TOTAL	26	85

ACTIVES IN TEST SET 2

175 compounds with confirmed T/C ≥ 120, including
32 compounds with T/C ≤ 175

RANK (Deciles)	NUMBER OF ACTIVES		
	T/C ≥ 175	T/C < 175 ≥ 120	
1 (lowest)	0	9	
2	0	7	
3	0	9	44
4	0	12	
5	0	7	
6	0	12	
7	0	8	
8	2	21	
9	6	18	
10 (highest)	24	38	
TOTAL	32	141	

4. The improvement obtained on novel actives was best demonstrated by running the new active training sets which excluded compounds in well-known chemically active classes through both models, i.e., (2A + C, including analogs) and (5A + C - analogs). The effectiveness of the two models are illustrated in Figures I and II. Each of these figures represent the percent active compounds that would be lost using varied cutoff points for acquisition based on activity scores calculated by the models. The scale used in Figure I was the distribution of scores derived by running test set 1 through each of the models. The scale used in Figure II was based on the results of test set 2 in both models. Figure I demonstrates that were a 50% cutoff established (i.e., selection of only those compounds with activity scores in the upper five deciles), the use of the (2A + C including analogs) model would lose 40% of the novel actives whereas with the (5A + C - analogs) model only 32% would be lost. Or, viewed another way, the (5A + C - analog) model permits going to a 60% cutoff for the loss of the same percentage of novel actives. Figure II indicates that the change in scale resulting from the use of data from test set 2 enhanced the improvement of the new model. At the 50% cutoff, the (2A + C including analogs) model lost 38% of the novel actives; at this same cutoff, the (5A + C - analogs) model lost 28%. In the definitive model which will be based on all the compounds tested in the P388 system through NSC 300000, the scale will be determined by the broadest range of compounds.

5. A solution to a long-standing problem in applying this method has been discovered. The problem is that weights determined by statistical significance tend to yield large numerical values for high incidence molecular fragments. Generally these have no relevance to activity, i.e., the benzene ring. The solution tried was to divide the weights of all features occurring more than ten times by the log to the base ten of their incidence. The resulting weights improved performance substantially on the test sets and also somewhat on the training set where no improvement was expected. This modification was incorporated into the new model, subsequent to the comparison in Figures I, II.

Proposed Course

1. The use of the model as an aid to selection will be evaluated over the next year. In order to be useful the scores must at least be relatively unpredictable, i.e., it should not be possible, after some experience, to predict routinely how the model would rate most compounds.

2. As more data from P388 prescreening are available, the model will be periodically changed by updating the training set to include these data.

3. Research will continue on methods to increase the specificity of the molecular fragment features and to avoid redundancy among the features. Experiments will be made to evaluate the utility of new fragments as features.

4. When demonstrated to be useful, the model will become one component of a three-part preselection subsystem to optimize the selection of

0 (2A + C, including analogs) model
* (5A + C - analogs) model

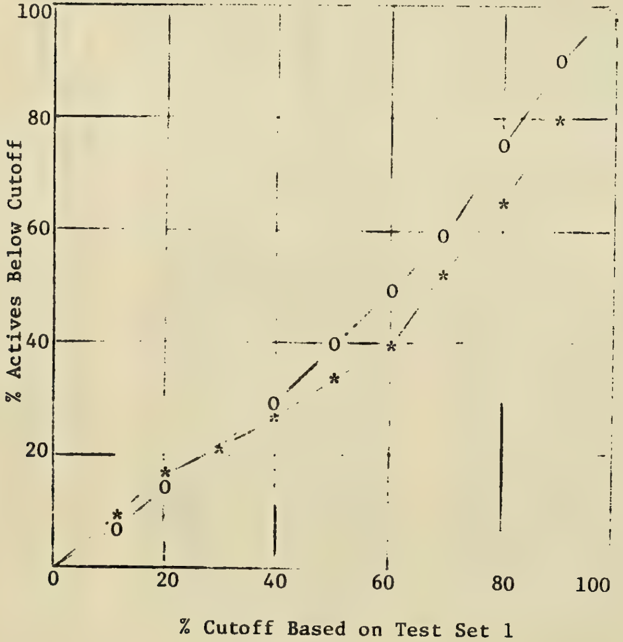


Figure I

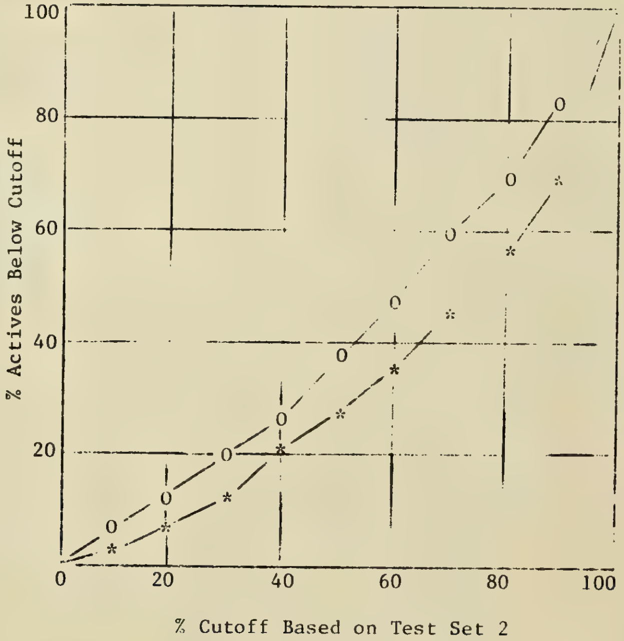


Figure II

acquisition. In addition to the information provided to the decision-maker by the model this subsystem will advise whether a potential acquisition: (a) is a duplicate or near duplicate of a previous accession; or (b) falls into a chemical class to be emphasized or deemphasized in acquisition.

5. When a sufficient number of structurally varied compounds which are active in the P388 prescreen have been tested in the DCT panel of screens, models will be developed and tested to optimize the testing of such compounds, e.g., when the supply of materials or the screening capacity is limited.

Significance for Biomedical Research and the Program of the Institute

1. The enrichment effect produced by preselecting compounds should increase the number of actives within the current screening level and maximize the potential of screening compounds with unique structural characteristics not adequately represented in the NCI collection.

2. The method developed for the prediction of probability of activity is now recognized as a contribution to the state of the art of broad range structure-activity relation work. It can be applied to many areas, e.g., using carcinogenicity or toxicity as the biological activity. The work was presented at regional and national meetings by the principal investigator on three occasions last year. Copies of the program and documentation have been requested by investigators in industry and academia. About 100 requests for reprints of the major publication to date have been received.

Publications

Hodes, L., Hazard, G.F., Jr., Geran, R., and Richman, S.: A Statistical-Heuristic Method for Automated Selection of Drugs for Screening. J. Med. Chem., 20: 469-475, 1977.

__________. A Statistical-Heuristic Method for Automated Selection of Drugs for Screening: Experiments with a Large Data Set. Submitted to J. Med. Chem.

SUMMARY REPORT OF THE NATURAL PRODUCTS BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT
October 1, 1977 - September 30, 1978

One of the major objectives of the Division of Cancer Treatment is to discover novel types of compounds with anticancer activity which can provide a basis for new chemotherapeutic agents.

In this regard natural product research has a long history of producing novel and unusual types of chemical structures which show many types of biological activity and indeed the basic structural types of various classes of natural products have led to development of many major classes of chemotherapeutic compounds. The investigation of natural substances for anticancer activity can provide new types of compounds for evaluation which possess unusual properties and can lead to new drug classes for chemotherapeutic evaluation in cancer treatment.

The Natural Products Branch has actively pursued acquisition, isolation, structure determination and testing of compounds from microbial, plant, and animal sources in order to obtain new leads for further development in the NCI program.

The major program areas of the Natural Products Branch include: 1) contract research directed towards isolation of new agents from active extracts of plant, animal (including marine organisms) and microbial sources; 2) worldwide procurement of new plants, marine organisms, and microbes for preparation of extracts and screening for anticancer activity; 3) procurement and preparation of large quantities of active agents for toxicological and clinical studies; 4) worldwide literature surveillance for the acquisition of natural products with demonstrated biological activity or novel structural types for evaluation.

Objectives

As part of the overall Division of Cancer Treatment Program, the Natural Products Branch has three major objectives:

I. Plant and Animal Products

The acquisition of new active antineoplastic agents from higher plants and from animal products (marine primarily) is the major function of this section. A contract program exists to obtain, select, and process enough plant material to obtain sufficient novel material for chemical identification and antineoplastic testing. Critical to this program is the ability to ensure a supply of plant material which is deemed of interest to NCI.

A contract program exists to obtain marine animal products in sufficient quantity to extract, isolate, and identify the active antineoplastic compounds. A new area to be exploited is the fermentation of plant cells as an alternate route to supply plant products and to study precursor yield improvement.

II. Fermentation

The acquisition of new antineoplastic agents from microbial fermentations is the primary function of this area. A contract program exists to isolate unique organisms and to systematically evaluate the microbial world for its potential to produce antineoplastic agents. The capacity exists in these contracts to produce sufficient amounts of material for clinical studies. The entire program is using novel methods to obtain unique organisms in the hope of generating numerous new structured leads. Pre-screens (*in vitro* tests) have increased the efficiency of the program and decreased the *in vivo* testing load for primary fermentations.

III. Worldwide Surveillance of Natural Products

This area of work is carried out by the in house staff. Literature, personal contacts with scientists, universities and industrial companies allows NCI to determine if any new natural products with various biological activities have been isolated and are worthy of testing in the National Cancer Program. Selected biological activities, new structure or organ site specificity determines if a compound should be obtained and evaluated in NCI tumor screens.

Program Implementation

The objectives of the Natural Products Branch are carried out through the Sections and the Office of the Chief and are:

I. Plant & Animal Products Section

The objective is the development of novel anticancer drugs from higher plants and animals (including marine organisms and insects). In support of this activity the Section maintains a worldwide program of acquisition of plant and animal materials. These materials are supplied by two collection contracts, purchase of new extracts and voluntary submission from natural products researchers. A contract also exists to prepare extracts of new materials for preliminary screening. Materials corresponding to active extracts are then recollected in quantities large enough for fractionation studies and fractionation is undertaken by one of six contractors who undertake the isolation, purification, and structural elucidation of the active substances in the extracts. There are four contractors involved in isolation of novel antineoplastic agents from plant materials and two contractors primarily involved with animal extracts. These contractors also reisolate larger quantities of pure compounds for further screening as required. A preparative scale isolation laboratory is available to produce large amounts of pure compounds for toxicology and clinical studies.

The Section also works closely with scientists worldwide who are doing isolation studies of plant and animal materials. This work includes arranging for testing of samples, monitoring progress of isolation studies and lending assistance in natural products research in the anticancer area as needed. Their non-contract research is funded by NIH grants, PL 480 funds, or grants from universities or foreign governments.

II. Fermentation Section

The objective is the isolation and development of novel antineoplastic drugs derived from microbial fermentations. In support of these tasks, the Section maintains six contracts to isolate unique organisms, and to systematically evaluate the microbial world for its ability to produce novel antineoplastic agents. The Section maintains four contracts to isolate, identify, and scale-up active materials of interest for NCI evaluation. These antineoplastic agents are supplied in quantities sufficient for toxicological and clinical trials.

The Section directs programs in biotransformation, co-metabolism and in the production of interferon from lymphoblasts. This work includes fermentation, development, and chemical isolation and genetic and culture research. More than 5,000 organisms are fermented/yr and evaluated for their ability to produce novel antineoplastic agents. Two contracts are devoted to biotransformation of known structures in order to increase the activity, lower toxicity, or in general, improve the basic structure as an antitumor agent. This area has been quite productive and several of the large pharmaceutical contracts systematically do biotransformations with some of their unique compounds.

III. Worldwide Surveillance

This activity is carried out by the Branch and is important in the obtaining of novel natural products for evaluation in the program. The maintenance of worldwide contacts with scientists or industrial companies and universities is essential to obtain as early as possible any novel compounds with biological activity. These compounds are reviewed for interest and if deemed interesting are tested in the NCI program.

Branch Staff

The Natural Products Branch is composed of a staff of seven persons consisting of three senior professional scientsts (GS-13 or above), one other professional staff member and three technical and clerical staff.

The responsibility for the activity of the Branch is as follows:

Natural Products Branch
Chief - Dr. John D. Douros
    Plant and Animal Products Section
    Head - Dr. Matthew Suffness
    Fermentation Section
    Head - Amelia Acierto

Program Operation

Fermentation Section

The fermentation program has been markedly changed during the last three years to hopefully obtain more novel organisms which will produce more novel antineoplastic agents. In order to cut costs in the *in vivo* testing of primary fermentation broths, more use has been made of pre-screens to select fermentations for *in vivo* testing. Although not ideal, the pre-screens used are Infectious Bronchitis virus test (1 contract), cell cytotoxicity (KB, LE, PS by 3 contractors),microbial mutants (3 contractors), enzyme inhibition (1 contractor, and phage induction (2 contractors). The results of using a pre-screen allowed NCI to decrease the number of primary fermentations to be tested *in vivo* from 5,011 to 1,347 with an overall increase in the number of actives (due to different organims being isolated). The fermentation program has made a marked effort to obtain novel organisms never before screened for their antineoplastic activity by using unusual substrates (hydrocarbons, terpenes, polyhydroxy acids, mixed substrates, etc.) and varied culture isolation techniques (sprinkle plate, percolation, soil feeding, pollen baiting, etc.). Results shown in Table 1 indicate that it was a highly productive year in obtaining new antineoplastic agents. However, the real benefits of the program will not be known until one sees how many of the new compounds are clinical candidates. We presently have 66 new fermentation compounds in the tumor panel and 133 compounds undergoing special testing in various phases of the panel.

TABLE I

1977 FERMENTATION CONTRACT PROGRAM RESULTS

Contractor	Cultures Isolated	Pre-Screens Active In Vitro	(a) Active *In Vivo* (PS leukemia)	Undergoing Chemical Fractionation	Novel Antineoplastic Agents Isolated
A	1,347	360	22	3	2
B	2,471	655	44	33	9
C	841	154	49	49	1
D	352	378	23	14	0
	5,011	1,347(26.8%)	138(10.2%)	99	12

(a) Normally only 1% of the cultures isolated produce *in vivo* active fermentation broths and all the *in vitro* actives have not been evaluated *in vivo* as yet.

Biotransformation and cometabolism are two techniques also being evaluated for their potential to produce interesting microbial metabolites. Several contractors are systematically evaluating the microbial world to attempt to identify which microbial type is most productive in producing novel antineoplastic agents as well as examining some of the microbes as immunostimulants. Biotransformation and the systematic evaluation of microbes are designed to increase the yield of antineoplastic agents per number of fermentations required.

The biotransformation program includes microbial and chemical modification of compounds which have a structural nucleus of interest to NCIbut the drug itself is either too toxic, has some ill-desired side effect, or is not quite active enough. It is hoped that with subtle select modifications made microbially, a better compound will be obtained. Already eight such compounds have been obtained this year and testing is being conducted to see if they will be candidates for toxicology. An analog program as described above along with chemical modifications produces new compounds which may act by the same mechanisms of action as the parent compound, but may give some beneficial factor such as less toxicity, more activity, or better distribution.

TABLE 2

NCI BIOTRANSFORMATION SUMMARY

Cultures	Substrates to be transformed	Biotransformation Products	Novel Antineoplastic Agents Isolated
108	Emetine	3	-
61	Camptothecin	2	-
42	Anguidine	6	3
90	Mitomycin	2	-
90	Rhodomycinone	3	-
70	Sterigmatocystin	2	-
22	Marcellomycin	2	-
90	Mithramycin	14	-
190	Ellipticine	10	3
15	Daunomycin	3	-
25	Streptonigrin	2	-
40	Thalicarpine	5	-
15	Nogalomycin	3	2
		58	8

Co-metabolism is used to obtain completely new compounds and is a fermentation technique which allows one to short circuit the normal microbial metabolism causing unusual compounds to be formed. This work has not as yet been initiated due to the limited funds available.

Only fermentation broths which show in vivo activity are chemically fractionated and the active compounds isolated.

Table 3

FERMENTATION LEADS

	T/C 125-149	T/C 150-174	T/C 175-199	T/C 200+	Total
Bristol	-	-	-	7	7
Michigan Health	104	133	58	53	348(a)
Parke-Davis	16	11	5	8	40
Upjohn Company	30	20	5	4	59
W. R. Grace & Co.	56	25	4	6	91
Litton Bionetics	16	8	1	2	27
	222	197	73	80	572

(a) Probably 70% are knowns as they do presumptive chromatography later.

Biological test systems are used to monitor the compound isolation; thus by the time the final product is isolated, purified, and prepared for in vivo testing, as many as 500 tests containing this material have been evaluated. The amount of prior testing allows for greater insight into the handling and testing of the compounds and also a greater assuredness of the reproducibility of the material as a true active. In some cases only the in vivo assay shows activity which makes isolation a very laborous process.

TABLE 4

FERMENTATION CONTRACTS

CONTRACTORS	INVESTIGATORS	CONTRACT NO.
Bristol Laboratories	Bradner	N01-CM-77147
Bristol Laboratories	Claridge	N01-CM-43759
Georgetown University	Katz	N01-CM-43732
W. R. Grace & Co.	Calton	N01-CM-67074
Litton Bionetics (Project 17)	White	C02-5423
Michigan Department of Health	Schuurmans	N01-CM-33702
Microbial Chemistry Research Foundation	Umezawa	N01-CM-57009
Parke-Davis & Co.	Dion	CM7-7146
Rutgers University	Nickerson	N01-CM-67106
Upjohn Company	Neil	N01-CM-77100
University of Iowa	Rosazza	N01-CM-77176

Plant and Animal Section

The operation of the plant products area is divided into three main areas; procurement, preparation of extracts for screening, and isolation studies of active extracts.

The initial procurement of plants comes from two major sources, an interagency agreement with U.S.D.A. and a contract with the University of Hawaii. Other plant samples are procured by purchase and by voluntary submission from interested persons worldwide. Plant submissions are screened to eliminate excessive duplication of species already tested. The preparation of plant extracts for screening is performed under a service contract by Wisconsin Alumni Research Foundation.

Approximately 3-5% of the extracts tested show reproducible activity in one of the two screens used (P-388 and KB) and from these a selection is made of which plants should be collected in large quantities for isolation studies. This selection is based on the level of activity of the confirmed extracts and phytochemical knowledge of the constituents of the genera and species showing activity. Much of this chemical constituent data is obtained through a computer survey at the University of Illinois and is acquired on an individual genera basis.

Those plants selected for isolation studies are collected in 300-500 pound quantities and sent to one of three active contract laboratories for fractionation work. The progress of fractionation of the plant extracts is monitored by testing fractions in the PS or KB system and pursuing isolation studies only on the active portion of the fractionation materials. Fractionation is continued until the active materials are isolated in pure form. The purified materials are tested thoroughly and screening data is closely monitored by Natural Products Branch staff. Structural studies are performed on active compounds by the fractionation contractors. The production of active novel compounds from this approach has been satisfactory.

The animal products area is handled in similar fashion except that the isolation contractors are also responsbile for initial collection and preparation of extracts of new organisms as well as fractionation. The main emphasis in this area is on marine organisms which is a relatively new area and should lead to discovery of novel active compounds unrelated to those from the plant or microbial areas. Initial compounds indicate that the marine area will indeed yield completely different compounds than those found in the terrestrial world.

When compounds isolated from the above studies are required in large quantities the material is procured and sent to a pilot plant contractor for extraction and compound isolation.

TABLE 5

PLANT AND ANIMAL PRODUCTS CONTRACTORS

CONTRACTORS	INVESTIGATORS	CONTRACT NO.
Agriculture, U. S. Dept. of	Perdue	Y01-CM-40001
Arizona State University	Pettit	N01-CM-67048
Central Drug Research Institute	Dhar	PL-4-8001
Hawaii, University of	Sagawa	N01-CM-33747
Houston, University of	Weinheimer	N01-CM-87209
Illinois, University of	Farnsworth	N01-CM-67090
Monsanto Research Corporation	Beemsterboer	N01-CM-77140
Oklahoma, University of	Schmitz	N01-CM-67108
Polysciences, Inc.	Boettner	N01-CM-77070
Purdue University	Cassady	N01-CM-67091
Research Triangle Institute	Wall	N01-CM-67089
Virginia, University of	Sneden	N01-CM-67002
WARF Institute	Dennis	N01-CM-43707

Worldwide Surveillance

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries. grantees. academic institutions, research organizations,and other government laboratories to maintain our knowledge of natural products research and development throughout the world. Also this liaison allows us to obtain prime compounds soon after discovery for evaluation in the NCI program.

This year 452 new compounds were obtained in the program. Two hundred ninety eight were obtained because of this liaison, many which are active and are being obtained in larger amounts for evaluation. Close contact is maintained with the suppliers in order to provide interchange of chemical and biological data which is essential to insure efficient use of material.

A one week International visit to Europe and three weeks to India made direct contact with the following organizations:

India - All India Institute of Medical Sciences
Central Drug Research Institute
Cancer Research Institute, Tata Memorial Center
Haffkine Institute
Indian Institute of Technology
Hindustan Antibiotics, Ltd.
National Chemical Laboratory
Postgraduate Research Center
National Institute of Oceanography
Indian Institute of Sciences
University of Madras

Europe - Gruppo Lepetit Co.
Farmitalia
Monte Edison
Archifar Co.
Leo Pharmaceutical
Royal Danish School of Pharmacy

Many natural products are obtained from all parts of the world and from all types of organizations. The following criteria are used to determine if NCI is interested in testing such materials or in requesting them.

1. Compound has some anticancer activity vs any tumor whether in NCI's system or not.
2. A novel structure never evaluated in our program.
3. Activity vs yeast, fungi, or certain bacteria.
4. Reported antiviral activity (human, animal, or bacteria).
5. Reported enzyme inhibition of proteases, lipases, CAmp phosphodiesterase, aminopeptidase, transmethylase, etc.

Accomplishments

The Natural Products Branch provided many new compounds to the program (Table 6). These materials were of fermentation, plant, and animal origin. Fifty-eight of these materials have demonstrated confirmed activity in one or more of the primary screens (Step III). Five compounds have passed Decision Network II and are candidates for preclinical toxicological evaluation. They are Bouvardin (259968) and Taxol (125973) which are plant products and Aphidicolin (234714), Largomycin (237020), and Echinomycin (526147) which are fermentation products.

Of the 452 crystalline natural products added to our program, 154 (34%) were obtained from contracts and the remainder were obtained through worldwide surveillance.

The Branch manages a total of 23 contracts with industrial, academic, and research institutions, both domestic and foreign, and one contract at FCRC.

In summary, the following was achieved in the last 12 months by the Natural Products Branch:

1. A total of 7,727 new substances were obtained for screening.
    a. 1,347 fermentation products
    b. 227 animal products
    c. 6,153 plant products
    d. 452 crystalline materials

2. A total of 154 compounds were isolated and purified under contract.
    a. 46 fermentation products
    b. 108 plant and animal products

3. A total of 298 compounds were obtained from worldwide surveillance.
    a. 98 fermentation products
    b. 200 plant and animal products

4. Five new substances were assigned to Decision Network IIA (Table 7)
    a. 3 fermentation products
    b. 2 plant products

5. Twenty-three contracts were supervised by the Branch (Tables 4 & 5).
    a. 11 fermentation
    b. 2 plant collection
    c. 4 plant fractionators
    d. 3 animal
    e. 2 plant prep laboratories
    f. 1 plant extract

TABLE 6

MATERIALS ACCESSIONED AND ACTIVITY REPORT

	Extracts 1976-1977		No.Actives 1976 - 1977		Crystalline Material Submitted		Step III[1] 1976-1977		Dec. PT.II[2] 1976 - 1977		INDS filed 1976-1977	
					1976	1977						
Fermentation Products	2,518	1,347	92	138	217	205	19	29	4	3	0	1
Animal Products	1,537	227	90	52	39	62	0	1	0	0	0	0
Plant Products	8,673	6,153	567	807	140	185	39	19	1	2	0	2

1. Showing confirmed activity

2. Selected candidates for pre-clinical toxicological evaluation and clinical trials

TABLE 7

COMPOUNDS PASSING DECISION NETWORK IIA

NSC#	COMPOUND NAME	SOURCE
234714	Aphidicolin	Fermentation
237020	Largomycin	Fermentation
526147	Echinomycin	Fermentation
125973	Taxol	Plant
259968	Bouvardin	Plant

The major accomplishments were:

1. Successful production of kilogram quantities of daunomycin at FCRC.
2. Cost effectiveness of pre-screening fermentation broths.
3. Initiation of systematic evaluation of microbes for their ability to produce antineoplastic agents.
   a. Arthrobacter - complete
   b. Rhodococcus - underway
4. High number of novel compounds isolated from natural products contracts this year.
5. A banner year in obtaining novel natural products from our world-wide surveillance program.
6. A multitude of novel organisms being isolated from unusual substrates.
7. Liaison established with companies from Europe and India.
8. A successful Natural Products Contractors meeting in North Carolina.
9. Biotransformation chemical research has led to two interesting leads from Nogalomycin.
10. Many new anthracyclines and trichothenes produced by fermentation have entered our program.
11. The possibility of obtaining Maytansine much more cheaply by fermentation - chemical conversion has presented itself to our Branch. If the negotiation is successful it will amount to approximately $1 million savings.
12. An agreement with Smith, Kline and French to supply FCRC with unique actinomycetes with biological activity has been consummated.

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Patents

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SUMMARY REPORT OF THE DRUG EVALUATION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT
October 1, 1977 - September 30, 1978

The Drug Evaluation Branch (DEB) mission is to develop and coordinate laboratory projects for the recognition, recommendation, and pre-clinical development of improved therapies against human cancer. This is achieved by (a) screening large numbers of synthetic and natural products for biological activities suggestive of clinical utility; (b) detailed testing of materials of interest reported from other testing programs and in published literature; (c) evaluation of "active" drugs for conditions of usage which may optimize efficacy - e.g., modifications of route and schedule of treatment, combination chemotherapy, and combined treatment modalities; (d) research aimed at devising improved methods for pre-clinical drug evaluation; and (e) developing the resources - e.g., animals, computerized data processing and retrieval, and ancillary services - required for conduct of the Program.

Implementation is through contracts with qualified institutions. At the end of April 1978, DEB directed 60 contracts funded at approximately $19,000,000 (Table 1). An analysis of funding and a brief description of tasks performed under these contracts are in the report of the Director, DCT.

DEB includes the Office of the Chief (J.M. Venditti), the Automated Information Section (AIS) (J.F. Waters, Head), the Mammalian Genetics and Animal Production Section (MG&APS) (J. Mayo, Head), and the Screening Section (SS) (B.J. Abbott, Head); and is staffed by 12 senior professionals, two junior professionals, and 12 technical and clerical personnel. Staff functions to (a) determine scientific approaches to realization of mission objectives; (b) devise appropriate experimental protocols; (c) implement program by generating project plans, requests for proposals, and contract summaries; (d) review proposals and participate in source selection; (e) serve as contract project officers to insure scientific and technical sufficiency of contractor performance; (f) assimilate, review, and summarize laboratory testing results; (g) present potentially improved therapies against human cancer to the Associate Director, DTP, the DCT Decision Network (DN) Committee, and clinical groups; (h) present major findings to the scientific community through publications; and (i) prepare final reports of pre-clinical therapeutic efficacy of new drugs for inclusion in Investigational New Drug Applications (INDA) and clinical brochures.

The screening plan depicted in Chart 1 was facilitated by the successful development of animal-tumor systems presumed to be models of major human tumor classes and fundamental studies demonstrating the utility of human tumor xenografts for chemotherapy studies. It represents one arm of a prospective study of "animal-man" correlations; the other being the clinical trials of the drugs uncovered. Thus, we have been testing a limited number of synthetic compounds and "active" isolates from natural products against transplantable colon, breast, and lung tumors of mice; human tumor xenografts of these tumor types grown in athymic mice; and mouse tumors of known value (e.g,, L1210 and B16 melanoma). Prior to the implementation of this broad spectrum screen, only one or two animal tumor models were used. The greater effort required to test a material against most of the tumors in the spectrum plus the increased number of models, limited the number of materials that could be screened in the tumor spectrum to 500-1,000 per year. To select, from the thousands of materials submitted to this Program each year, those warranting full tumor spectrum testing, we have utilized an initial in vivo pre-screen, mouse leukemia P388, for reasons that were described previously. Although cost limitations necessitated the use of the pre-screen for selecting most of the materials to be accorded full spectrum testing, we recognize that no single model is known to predict perfectly for clinical utility. Thus, input to the spectrum is augmented with materials of interest based on activities in other screening programs and selected biochemical and biological assays.

Crude natural products are screened against mammalian cells in culture and in vivo against P388 as well. When activity is observed in both, the less costly cell culture system is used as the primary bioassay tool for following activity through fractionation procedures to isolate the active antitumor component. Most synthetic compounds and crude plant extracts enter the screen without previous biological data. In contrast, most fermentation products screened in vivo are selected from microbiological or biochemical assays or are products of microbial biotransformation of known active drugs. These materials, when purified, may be tested against the tumor spectrum regardless of effects against P388. As materials emerging as active from any of the spectrum models are subjected to adequate clinical trial, this approach to screening will provide, prospectively, critical information relative to the value of the animal tumors used individually or in concert.

From April 1 through March 31, 1978, screening was initiated on a total of 23,934 materials (14,774 synthetic compounds; 6,256 plant products, 249 animal products; and 2,655 fermentation products); an approximately 30% reduction from the imput during the previous 12 month period. This resulted in only a 10% decrease in number of drug screening tests performed. A test is defined as an experimental group of from six to ten animals. That a reduction of input of materials was not reflected in a proportionate reduction in testing level can be attributed to a number of circumstances. First, many materials received in the 1976-1977 period were actually tested in the 1977-1978 period. More importantly, the decrease in input had its heaviest impact on the P388 pre-screen where the number of tests per materials is minimal because only one tumor model is involved. Of greater significance was the increase in tumor panel testing from 13% of the total in vivo testing in 1976-1977 to 28% of the total in 1977-1978. In addition, 1,319 materials were added to the Selected Agents Listing (SAC) in the 15 month period from January 1, 1976 to March 31, 1977 while 1,395 were added in the ensuing 12 month period. This 25% increase in rate of additions to SAC is significant because most of such materials are scheduled for complete or partial testing against the tumor panel involving a number of tumor models for each material.

At the inception of the screening plan illustrated in Chart 1, it was estimated that an input of synthetic agents and natural products equivalent to that of the current reporting period would require tumor panel testing for up to 1,000 compounds per year. This capacity has been reached for the following mouse tumor models: B16 melanoma, colon tumor 38, leukemia L1210, and the Lewis lung carcinoma. We can now screen 750 compounds per year against the CD8F1 mouse mammary carcinoma.

Our capacity for screening against the human mammary, colon, and lung xenografts was expected to be sufficient for 375 compounds per year by July 1978. It should be noted that large scale screening against human tumor xenografts and breeding of the required numbers of "nude" mice had never been attempted prior to the inception of our current screening plan. The first contract for such screening was awarded in September 1976 when only 50 mice per week were available. Animal disease problems in both the breeding and testing laboratories hampered the development of the desired test capacity. However, the capacity to screen at a rate of 250 compounds per year was developed by December 1977 and the 50% increase expected by July 1978 is gratifying.

We can expect a continued shift in emphasis away from pre-screening to the tumor panel in the future. Although the input of materials into the panel is not expected to exceed 10% of the input into initial screening, panel testing per compound costs, conservatively, 30 times the cost of pre-screening. Consequently, the reduction in input of materials over the last few years has not been accompanied by a reduction in costs. It is fortuitous that we have been able to affect these substantial changes in the practice of screening with an essentially level budget even in the face of progressive economic inflation.

During this reporting period, the Data Review Subcommittee of the Drug Evaluation Committee (DEC) reviewed test results for 2,289 materials and referred 98 to the full DEC. Thirteen materials passed Decision Network 2A of the DCT Linear Array and were entered into development to clinical trial. The latter are listed in Table 8 of the current report of the Screening Section, DEB which also shows the date of assignment to DN2A and the experimental basis.

During the past year DEB summarized the antitumor data on the following drugs as part of the Investigational New Drug (IND) Applications for submission to the Food and Drug Administration: Pyrazolo-Imidazole (NSC-51143), Indicine N-oxide (NSC-132319), L-Alanosine (NSC-153353), Soluble ICRF-187 (NSC-169780), and PALA (NSC-224131). In addition, a summary was prepared on Spirogermanium (NSC-192965) as part of the IND Application from Georgetown University. Annual progress reports were filed on Dromostanolone (NSC-12198E), 3-Deazauridine (NSC-126849) and Rubidazone (NSC-164011).

A unique feature of DEB's activities is their magnitude. Its anticancer testing program is the largest drug screening operation in existence. Ancillary support activities; i.e., maintenance and operation of the computerized biological data file, and laboratory animal production - resources which are integral parts of the overall Branch function - are also the world's largest programs of their kind. In practicing management principles aimed at multiteam building approach to organizational development, the research and resource projects within the Branch are interdigitated to serve the targeted Program of DCT, NCI and the National Cancer Plan. Consequently, DEB is in a position to supply, to investigators world-wide, services (testing of materials antitumor screening data, experimental animals) which would be largely unattainable elsewhere.

The Automated Information Section (AIS) implements and maintains the biological data processing systems which provide for the storage and retrieval of data generated in testing laboratories. The data base can be queried by special programs and provides summary data for (1) review of antitumor activity, (2) evaluation of existing screens, (3) design and evaluation of new experimental models, (4) detailed evaluation of active drugs and drug combinations, and (5) information on utilization of resources and costs essential for efficient program management. During the past year, AIS processed 250 special queries from NCI staff and outside investigators. A major effort was accorded to revising the biological data file itself. The purpose of the revision is to maximize the information presented on the Screening Data Summary (SDS) computer output and was impelled by the use of progressively complex experimental protocols for screening and the inclusion of 18 new experimental models to the program. The revised system will be fully operational by September 1978. DEB's automated data processing systems are currently developed and operated by the Value Engineering Company (Contract N01-CM6-7086). Computer programs are developed by AIS staff in collaboration with the contractor. The principal biological data file is maintained by the Division of Computer Resources and Technology (DCRT).

Within the last two years, DEB developed two computerized files to maintain more complete and accurate records for compounds of special interest to DCT and for the Operating Committee compounds; i.e., those which passed DN2A and are in development to clinical trial. The Compounds of Interest (COI) or Transaction File was instituted because of the need to retrieve information from meetings, letters, personal communications and publications which could be of critical importance to Program decisions. Important features of the transaction file are that (1) any compound of interest can be entered, (2) there is no restriction on the type of information that can be entered, and (3) there is no restriction on search criteria. The system is maximally flexible and is designed to centralize information from diverse sources for ease of summarization; for example, when preparing reports for IND applications. The Operating Committee file is more structured. It deals only with compounds which have passed DN2A until investigational clinical studies are complete or until the compound is dropped from development. It replaced the manually generated master forms that kept the Operating Committee abreast of progress of compound development to assure that essential tasks (screening, formulation development, toxicology, INDA filing, etc.) were done in a logical and time efficient way. Both the COI and Operating Committee files were developed as a collaborative effort between the AIS and Screening Section staffs and the IIT Research Institute (Contract N01-CM4-3755).

In the last year, MG&APS supplied approximately 6,500,000 laboratory animals to support research activities carried out under DCT contracts, other NCI contracts sponsored mainly by the Division of Cancer Cause and Prevention (DCCP) and the Division of Cancer Biology and Diagnosis (DCBD), NIH intramural laboratories via the Veterinary Resources Branch, Veterans Administration (VA) Research Facilities, and research grants. In 1977, as a result of the internal reorganization of DCT, MG&APS came under the supervision of DEB, by far the principal user of this resource. At that time there was no precise mechanism for establishing the cost to Program for animals distributed to various users. Under its contractual agreements, DCT bears the cost of animals used in its contract program. However, there was no mechanism for reimbursements for animals distributed to other users. While the numbers of the latter are small compared with total production, they have become significant. This and rising costs have strained the budgetary allocations for this segment of Branch activities. During the present reporting period a number of steps were taken to place the animal production program on a sound fiscal basis. The cost per animal was established for each species and strain produced and for various types (e.g., weanlings, pregnants, pregnants with litters, ex-breeders, etc.) within each inbred and hybrid strain. These cost figures include the cost of operating the primary genetic centers, production centers, animal disease diagnostic and control centers, contracts devoted to studies of host tumor compatibility, support to the National Academy of Sciences for dissemination of information on breeding and care of laboratory animals, and operation of DEB's frozen bank of animal and human tumors which distributes tumors on a regular basis to contract testing laboratories and to other investigators. Arrangements were made for DCT to be reimbursed for animals distributed to DCCP, DCBD, and the VA. The extent to which we can continue to supply animals to grantees and possible sources of financial support for this service have not yet been established. However, DEB has given highest priority to a collaborative effort between MG&APS and AIS to develop computerized systems to account for all animals produced, to whom they are distributed, and their cost. These systems are expected to be fully operational during calendar year 1978. A preliminary study suggests that of a total non-reimbursed animal production expenditure of approximately $7,000,000 in FY 1978, at least 10% was expended for animals given gratis to grantees and non-NCI collaborators of NCI staff. While the percentage may be low, the amount is very close to the dollar overexpenditure for FY 1978.

During the past year DEB undertook the complete revision of the flow of logic relative to DCT Decision Networks 2A and 2B. It is the first revision since the mechanism was instituted in 1972. A tentative revision of DN2A has been completed and the revision of DN2B is expected to be completed during this reporting period.

Of the 13 materials passing DN2A from April 1, 1977 through March 31, 1978, nine were selected because of activity against one or more mouse tumors in the DCT panel, one because of activity against an estrogen dependent DMBA-induced rat mammary tumor and the MTW9 transplantable rat mammary tumor, one because of its radiosensitizing activity, one because of radioprotecting activity, and one (Thymidine) because of its activity against human tumor xenografts.

It has long been known that Thymidine can reversibly inhibit the progression of tissue culture cells through the cell cycle, inhibit ribonucleotide reductase, and interfere with DNA synthesis. Recently, the group at the Stehlin Research Foundation (Contract N01-CM6-7073) demonstrated that malignant human cells derived from melanoma and colon carcinomas were more sensitive to high levels of Thymidine than non-malignant human tissues. The contractor subsequently reported that very high dose continuous infusions of Thymidine inhibit several human melanomas and teratocarcinomas grown in nude mice. Inhibition of tumor growth in vivo was accomplished without toxicity to the host. The indication that Thymidine when given alone in very high concentrations may be an effective anticancer agent with a high therapeutic ratio makes it imperative that these observations be confirmed in an independent laboratory. To this end, DEB is in the process of having Thymidine tested against a spectrum of human tumor xenografts at the University of California at San Diego where 23 such tumor lines are being studied.

The Stehlin group is continuing to characterize human tumor xenografts with respect to growth characteristics, transplantability, in vitro growth, karyotype, ratio of human tumor cells to mouse cells, and is monitoring stability of individual tumors by isozyme profiles. Data sheets on all new human tumors developed as xenografts are submitted to DEB. These include the patients primary diagnosis, time of transplantation into "nudes" relative to diagnosis, patient treatment prior to presentation, further treatment and response, current number of passages in mice, change in xenograft growth pattern, percentage of "takes" in mice, extent of necrosis, maximum volume reached, metastases, sensitivity to known chemotherapeutic agents, and growth in vitro. The contractor is also collaborating in studies of correlations between responses of established SC xenografts and human tumors implanted under the kidney capsule of nude mice, using technique developed at the Mason Research Institute (Contract NO1-CM5-7030). In continuing studies of the sensitivity of human tumor xenografts to drugs of known clinical effectiveness, the Stehlin group tested six clinically effective drugs against one or more of 12 SC implanted tumors (four breast carcinomas, three colon carcinomas, one lung carcinoma, two melanomas, one liposarcoma, and one chondrosarcoma) as follows:

Adriamycin - 8 tumors
5-Fluorouracil - 9 tumors
Cytotoxan - 6 tumors
L-PAM 5 tumors
Methotrexate - 6 tumors
VCR - 1 tumor

Various patterns of activity were seen, but the most significant finding was that individual human tumors of the same histological type differ in response to the same drug. Four tumors (two breast carcinomas, one colon carcinoma, and one lung carcinoma) were treated with one or more of ten drugs in current development to clinical trial. Significant findings included the activities of Pentamethylmelamine (PMM, NSC-118742) and Hexamethylmelamine (HMM, NSC-13875) against a lung xenograft; DON against a colon xenograft; and AMSA (NSC-249992), Lymphosarcin (NSC-208642), Bruceantin (NSC-165563), and 5-Methyl-tetrahydrohomofolate (NSC-139490) against breast xenografts.

In screening against the human tumor xenografts selected for inclusion in the DCT tumor panel, Battelle Columbus (Contract NO1-CM6-7099) reported the following drug activities:

MX-1 (Breast Carcinoma)

Tumor Regression - NSC-163501 - AT125
NSC-56654 - Azotomycin

NSC-182986 - AZQ
NSC-7365 - DON
NSC-135758 - Piperazinedione
NSC-283162 - TMM

Tumor Inhibition - NSC-178248 - Chlorozotocin
NSC-224131 - PALA
NSC-118742 - PMM
NSC-752 - 6-Thioguanine
NSC-77213 - Procarbazine

LX-1 (Lung Carcinoma)

Tumor Regression - NSC-56654 - Azotomycin
NSC-7365 - DON

Tumor Inhibition - NSC-163501 - AT125

CX-2 (Colon Carcinoma)

Tumor Regression - NSC-7365 - DON

Tumor Inhibition - NSC-56654 - Azotomycin
NSC-208642 - Lymphosarcin
NSC-118742 - PMM

The Institute of Cancer Research, England (Chester Beatty Research Institute, Contract N01-CM4-3736) has successfully grown and carried by transplantation human bronchial, ovarian, colonic and renal carcinomas in mice artifically deprived of T-lymphocytes. All grew slowly and were shown to have a human karyotype even after several transplant generations. Responses to chemotherapy were similar to the expected clinical response with the important exception of Hexamethylmelamine (HMM) which caused complete regression of two lung carcinomas (even when up to 2.5gm in weight at the start of treatment) and a renal carcinoma, while completely inhibiting the growth of an ovarian carcinoma and a further lung carcinoma. Previously reported studies of the metabolism of HMM and screening of its more soluble demethylation products against a xenograft of the human epidermoid carcinoma, P246, led to the selection of Pentamethylmelamine (PMM) for development to clinical trial. During the present reporting period metabolism and physiological distribution studies of HMM and its metabolites has continued. Comparative toxicology of HMM and PMM has been completed. Contractor expected to receive clearance to proceed with clinical trial of PMM in May 1978. Contractor has continued studies to establish animal models using xenografts of human breast carcinomas. From 81 primary and six secondary breast carcinomas grafted subcutaneously to nude mice, ten transplantable cell lines have been obtained.

Compounds synthesized at the Institute of Cancer Research or submitted from sources in the United Kingdom were screened in one or more animal tumor models not used elsewhere in the DEB program. While contract funds are used to support a minimum part of this effort, the results are sent to DEB. In the last two years, emphasis was placed on comparing the antitumor activity of cis-Pt-II analogs synthesized in Great Britain. Highly active compounds have been found and these were transmitted to DEB for further testing. During the current reporting period the contractor has submitted results for 238 antitumor dose response tests; 86 against ADJ/PC6A, 92 against TLX/5 lymphoma, and 60 against various human tumor xenografts; and also submitted data on the activity of 52 compounds against W256 although DCT funds did not contribute to this work.

Recently (June 1978), the contract with the Institute of Cancer Research was augmented to place special emphasis on the following tasks: (1) validation of human tumor xenografts as models for cancer chemotherapy, (2) the use of selected human tumor xenografts for drug testing, (3) studies of the biochemical basis for success or failure in the treatment of human tumors, (4) small animal toxicology, and (5) Phase I clinical trials in Great Britain.

Accomplishments under multifaceted contract NO1-CM4-3756 with the Southern Research Institute (SRI) are too numerous and varied to include in this report except in a cursory manner. Emphasis was placed on studies of the animal tumors in the DCT panel, and these led to a number of refinements of experimental procedures. SRI evaluated 398 drugs against the panel. This required 4,845 tests against panel tumors, compared with 2,982 the previous year. Approximately 10,000 in vivo tests were conducted against the P388 pre-screen and other tumors. In general, studies relative to the DCT tumor panel covered three areas: (1) routine drug screening, (2) special testing using panel tumors, and (3) developmental studies to improve the quality of the panel models. The contractor developed a "target evaluation" model to determine relative activity of drugs against the panel tumors. In addition to in vivo testing, a total of 2,704 tests were conducted in vitro on 669 natural products and 74 synthetic compounds. Developmental research was conducted in the following selected areas:

1. The use of kinetic data in protocol design for screening studies of first-generation transplants of CD8F1 mammary carcinomas.

2. The kinetics of multiple modality therapy.

3. Variables in experimental tumor systems which complicate interpretation of data.

4. Evaluation of drug combinations against murine tumors.

5. Experimental models for human lung tumors.

6. Biology and response to drug treatment of transplantable C3H mammary adenocarcinomas.

7. Biology and therapeutic response of a mouse mammary adenocarcinoma (16/C) and its potential as a model for surgical adjuvant chemotherapy.

8. Chemotherapy of intrathoracially implanted C3H mammary adenocarcinoma.

9. Surgical adjuvant chemotherapy trials with mammary adenocarcinoma 16/C and colon carcinoma no. 26.

10. Comparison of therapeutic activity of Adriamycin analogues in transplantable colon and mammary tumors.

11. Therapeutic trials with 2-Fluoro-Ara-A (NSC-118218) against L1210 leukemia *in vivo*.

12. Studies of combinations of adenine arabinoside and inhibitors of its deamination.

13. Response of several experimental tumors to *N*-Phosphonacetyl-L-aspartate (PALA, NSC-224131).

14. Immunotherapy of murine tumors using *Corynebacterium parvum*.

15. Chronochemotherapy: further evaluation of applying circadian rhythm adaptations in the treatment of L1210 leukemic mice.

16. Development of new drug resistance tumor variants; studies of resistance, cross-resistance, and collateral sensitivity; studies of mechanisms of resistance and rates of its development.

Work under Contract NO1-CM5-3765 with Arthur D. Little, Inc. (ADL) included four major tasks: analog comparisons, combination chemotherapy, special studies in response to questions arising during new drug development, and studies of combined treatment modalities. During the past year, approximately 1,000 compounds falling into about 27 structural classes were examined in direct comparison with the parent drug according to standard protocols. In addition to confirming promising activity, analogs of special interest were subjected to more detailed evaluation. A number of anthracenediones were examined against IV leukemias, Adriamycin-resistant leukemias, and a variety of solid tumors. On the basis of this testing, ADL recommended that NSC-279836, a dihydroxy derivative, was superior against B16 melanoma, colon tumor 26, IV-L1210, and P388/ADR and was, therefore, a better candidate for clinical trial than NSC-287513, the compound of this class originally placed under development to the clinic. A number of nitrosoureas were tested against a spectrum of IC implanted tumors. PCNU (NSC-95466), a new nitrosourea under development, was found to be as good as, and in some models, superior to CCNU, which was better than either BCNU or methyl CCNU.

ADL evaluated about 80 two-drug combinations against a variety of animal tumor models. The following combinations of clinically active drugs proved to be highly synergistic: L-PAM plus 5-fluorouracil (5-FU), nitrogen mustard plus Hydroxyurea, Mitomycin C plus Adriamycin and Mitomycin C plus Methotrexate. The combination of Actinomycin D and DTIC, as well as *cis*-Pt-II and DTIC, looked promising and may have relevance for the treatment of human melanomas and sarcomas. Thymidine potentiated the therapeutic effect of 5-FU against P388. The suggested protective effect of DL-α-tocopherol (Vitamin E) on Adriamycin toxicity in mice was also examined. Although there was a trend for Vitamin E to protect against the acute toxicity of Adriamycin, after the first

14 days the effect was diminished. Over the past year, the contractor also attempted to foster closer contact with the Cancer Therapy Evaluation Branch of DCT, the Sidney Farber Cancer Institute, and the Southeastern Cancer Study Group for clinical input in setting up combination chemotherapy experiments.

ADL participated in the evaluation of the M5076 mouse ovarian tumor, previously developed for screening at the Mason Research Institute (Contracts N01-CM5-7030 and N01-CM5-7003), as a possible addition to the DCT tumor panel.

Combined modality studies were devoted to the interactions of drugs and radiation in vivo. A number of hypoxic cell sensitizers of the nitroimidazole class and several cytotoxic agents, mostly aziridines, were examined in combination with radiation (colbalt source) against P388. Recently, ADL reduced its emphasis on the P388 model and attempted to develop new solid tumor protocols according to the "Treatment Linear Array for Radiosensitizers" as set forth by the Division of Cancer Treatment. Using the SC Lewis lung, the SC B16 melanoma, and the SC EMT6 murine tumors, which are part of the DCT panel for radiosensitizers, three new assays were being developed: cell survival, growth delay, and the tumor cure dose (TCD_{50}).

During the past year, the Catholic Medical Center of Brooklyn and Queens, Inc. (Contract N01-CM6-7081) tested about 120 compounds for antitumor activity using first generation transplants of the CD8F1 mouse mammary carcinoma. From these and other screening studies, particularly against the IP B16 melanoma and the SC Lewis lung carcinoma, PALA (NSC-224131), a transition-state inhibitor of aspartate transcarbamylase, was selected to go into clinical trial. In addition to these screening tests, some testing was also carried out in mice bearing spontaneous tumors. Three host defense modulators were evaluated alone and as adjuvant therapy. Of the three, which included Levamisole, Thymosin, and C. parvum, only C. parvum appeared to have significant antitumor activity in mice bearing spontaneous tumors. However, when either Phenylalamine mustard or 5-Fluorouracil-Thymidine was added to the regimen, a better therapeutic effect was achieved than with C. parvum alone.

The highlight of the research effort at CMC was the continuing progress on the 5-Fluorouracil-Thymidine combination in the treatment of CD8F1 first generation transplants. Last year it was observed that this combination gave a better antitumor response than 5-FU alone. Similar enhancement was observed with colon tumor 26. However, increased toxicity in the form of weight loss and early deaths was often noted in the groups receiving the combination. During the past year special effort was directed toward overcoming this toxicity by investigating various doses and schedules of administration of each drug and by adding other agents to the combination. The addition of Testosterone propionate and Uridine to the 5-FU-Thymidine combination alleviated some of the toxicity, but also reduced some of the antitumor effectiveness of the combination. Later it was discovered that by adding PALA to the 4-drug combination (5-FU, Thymidine, Uridine and Testosterone) that good tumor inhibition was achieved at tolerable toxicity to the host. In collaboration with Dr. Sol Spiegelman of Columbia University, a biochemical analysis of the interaction of 5-FU and Thymidine both in mammary tumor tissue and in normal host tissues was undertaken. Preliminary data suggested that in the presence of Thymidine, more 5-FU was diverted into RNA. This diversion is considered to be the biochemical basis of the therapeutic superiority of the combination.

Based on these laboratory findings, clinical evaluation of the 5-FU - Thymidine combination was initiated in 1977.

The objective of Contract N01-CM5-7029 with Yale University is to design and synthesize new antitumor agents which intercalate with nucleic acids. All of the compounds synthesized have been acridine derivatives and the molecular mechanism of their association with DNA has been evaluated as well as their effect on P388 leukemia in vitro and in vivo. Biochemical studies on the mechanism of action of the diacridines was done under grant support. It was shown that although the diacridines bind tightly to DNA and inhibit RNA synthesis, DNA synthesis and cell growth in vitro, these effects do not correlate with antitumor activity in vivo. However, recent findings showed that a significant inverse correlation exists between the percentage increased life span and the ability of the diacridines to enhance the rate of agglutination of P388 cells by wheat germ agglutinin. Diacridines which enhance agglutination fluoresce upon interaction with isolated plasma membrane of P388 cells, whereas those which inhibit agglutination do not fluoresce, although they do interact with the membrane. It is possible that the two groups of diacridines react with different regions in the cell membrane. Studies are being extended to characterize this membrane-related effect.

The purpose of Contract N01-CM6-7068 with Yale University is to screen new antifols for inhibition of human dihydrofolate reductase (DHFR), and to select promising agents for development toward clinical trial based on biochemical and/or pharmacological findings. In the past year, 14 new antifols have been so screened. In addition, several quinazoline compounds have been tested for their ability to inhibit DHFR and thymidylate synthetase. A number were tested against L1210 cells in culture. Of particular interest, is a thioquinazoline derivative which binds to the thymidylate synthetase FdUMP complex.

Studies have continued with NSC-249008, a quinazoline folate antagonist which previously has been shown to concentrate in human leukemia cells to a much greater degree (10-100 fold) than Methotrexate (MTX), and which exhibits a wider spectrum of activity against murine tumors than MTX. Investigations on the transport of NSC-249008 into L1210 cells showed only partial competition with MTX or reduced folates. Analytical methods for the quantitation of NSC-249008 are being studied so that its pharmacology in animals can be examined.

The cis-Pt-II (NSC-119875) Analog Coordinating Group continued to function under the Chairmanship of a DEB senior staff member. If pre-clinical activity and toxicity testing proceeds on schedule and if superiority of an analog over a parent drug can be demonstrated, at least one derivative should be ready to present for DN2A consideration by early Fall, 1978.

The Applied Mathematics and Data Analysis Project (ADL, Contract N01-CM3-3727) continued to utilize screening data from all appropriate contractors to define criteria for activity and toxicity in recently instituted animal screens, analyze variance among screeners, and to analyze the influence of experimental variables (host strain, number of mice per test and control groups, treatment schedules, etc.) which modify the reliability of results. In addition, the contractor, using continuously updated animal and clinical data, has applied multivariate statistical methods to develop discriminant functions or mathematical models that yield the "best" prediction of presumptive clinical activity against human tumors.

Refinement of this approach to assessing the relative contribution of each of a set of animal systems to selection for the clinic, will facilitate judgments regarding relative usefulness among our new animal tumor screens. Specifically, the objectives of this study of "animal-man" correlations are to determine (a) the human tumor(s) most likely to respond to new active drugs, (b) human tumors which are likely responders to older drugs which have not been adequately evaluated against them, (c) animal tumor models which contribute little to the prediction formula for a specific human tumor, and (d) animal models which closely simulate each other. We envision that the further development and use of such analytical procedures will result not only in improved clinical therapy, but also in a more efficient use of clinical and laboratory resources. While no single experimental model, in vivo or otherwise, predicts perfectly and absolutely for activity against human cancer, we recognize that no alternative to the use of non-human experimental systems exists. Therefore, it is incumbent on us to select models that provide the best tools for realization of our objective. Antitumor drug evaluation is a dynamic undertaking; the experimental models used in this program have changed considerably over the years, and we expect that they will continue to improve progressively.

CHART 1

FLOW OF DRUGS THROUGH DCT SCREENS

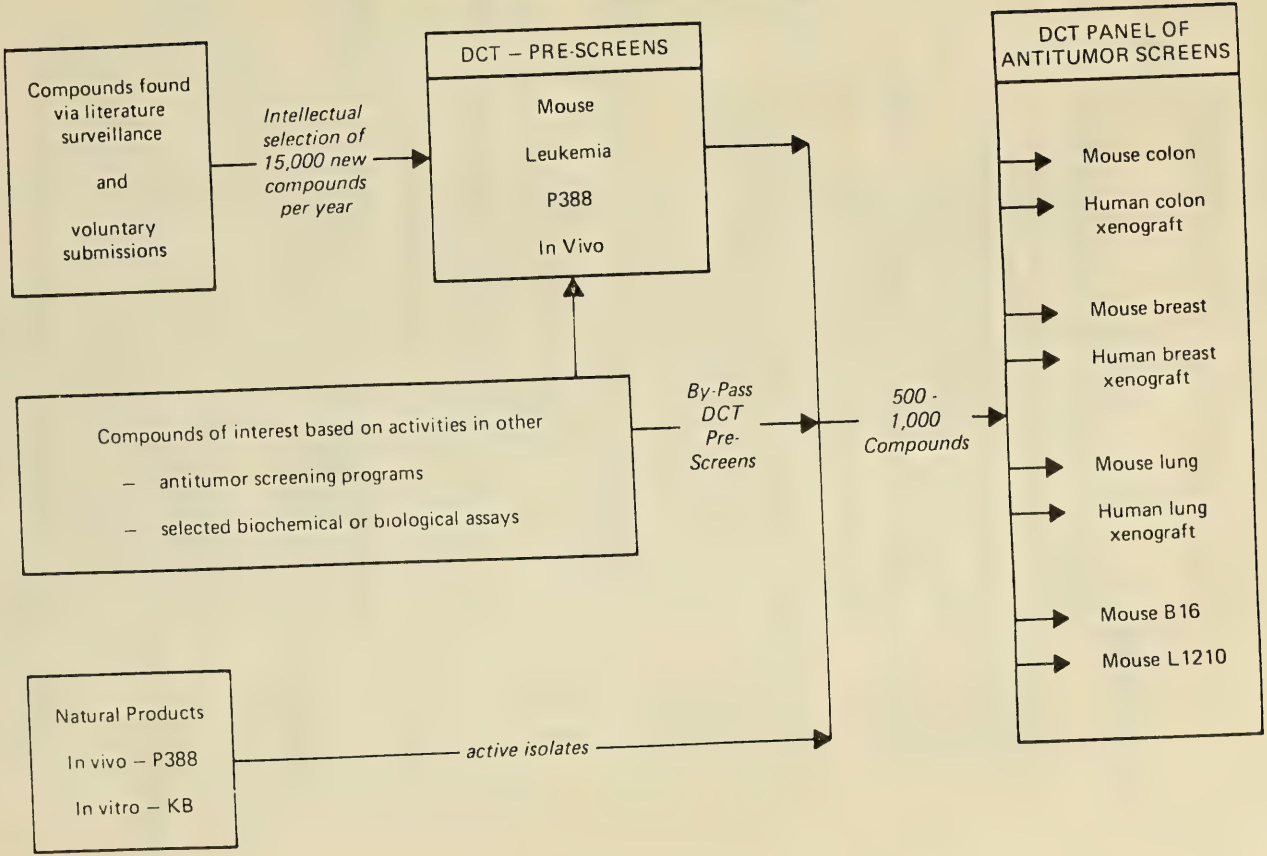

TABLE 1

Contracts Monitored by DEB During FY 1978

Contract Number	Institute
	1. In Vivo Screening Against Animal Tumors
5-7006	Arthur D. Little, Inc., Cambridge, MA
5-7005	Battelle Columbus, Columbus, OH
5-7007	Hazleton Laboratories, Inc., Vienna, VA
5-7004	IIT Research Institute, Chicago, IL
5-7040	Institute Jules Bordet, Brussels
5-7003	Mason Research Institute, Worcester, MA
5-3770	Raltech Scientific Services, Inc., Madison, WI
	2. In Vitro Screening Against Mammalian Cells
6-7078	Arthur D. Little, Inc., Cambridge, MA (terminated)
6-3832	IIT Research Institute, Chicago, IL
6-7075	Southwest Foundation for Research and Education, San Antonio, TX
6-7077	University of Miami, Miami, FL
6-7076	University of Wisconsin, Madison, WI
	3. Development and Use Of Human Tumor Xenograft Models for Drug Evaluation
6-7099	Battelle Columbus, Columbus, OH
6-7073	Stehlin Foundation for Cancer Research, Houston, TX
	4. In Vivo Screening of Analogs, Combination Chemotherapy and Chemotherapy Plus Radiation
5-3765	Arthur D. Little, Inc., Cambridge, MA
	5. Recognition, Evaluation, Pre-clinical Development of New Improved Anticancer Therapies; Associated Model Development
4-3756	Southern Research Institute, Birmingham, AL
	6. Pre-clinical and Clinical Evaluation of Anticancer Agents
4-3736	Institute of Cancer Research, London
	7. Screening Against Murine Mammary Tumors
6-7081	Catholic Medical Center of Brooklyn and Queens, Inc., Queens, NY

TABLE 1, CONTINUED

Contracts Monitored by DEB During FY 1978

Contract Number	Institute
	8. Collection and Screening of European Anticancer Agents
3-3720	Mario Negri Pharmacological Institute, Milan
	9. Collection and Screening of Indian Plant Products
PL-48002	Indian Cancer Research Institute, Bombay
	10. Development and Operation of DTP Biological Data Processing System
6-7086	Value Engineering Co., Alexandria, VA
	11. Services in Support of Screening Program
4-3755	IIT Research Institute, Chicago, IL
	12. Applied Mathematics and Data Analysis
3-3727	Arthur D. Little, Inc., Cambridge, MA
	13. Biochemical Studies (two contracts to be terminated in June 1978)
5-7029	Yale University, New Haven, CT
6-7068	Yale University, New Haven, CT
	14. Operation and Maintenance of Bank of Frozen Animal and Human Tumors
8-7163	Mason Research Institute, Worcester, MA
	15. Primary Genetic Centers to Insure Genetic Integrity of Breeding Stock for Production of Experimental Animals
5-3813	ARS/Sprague-Dawley, Madison, WI
7-7141	Charles River Breeding Labs., N. Wilmington, MA
7-7168	Harlan Industries, Cumberland, IN
7-7165	Leo Goodwin Inst. for Cancer Research, Fort Lauderdale, FL
7-7166	Simonsen Laboratories, Gilroy, CA

TABLE 1, CONTINUED

Contracts Monitored by DEB During FY 1978

Contract Number	Institute
	16. Production of Pure Strains of Inbred Mice
5-3846	ARS/Sprague-Dawley, Madison, WI
5-3848	Charles River Breeding Labs., N. Wilmington, MA
5-3775	Laboratory Supply Co., Indianapolis, IN
5-3847	Simonsen Laboratories, Gilroy, CA
5-3845	Southern Animal Farms, Prattville, AL
5-3818	Microbiological Associates, Inc., Bethesda, MD
	17. Production of Hybrid Mice for Antitumor Drug Evaluation
5-0583	Bellaire Acres, Danville, IN
5-0598	Charles River Breeding Labs., N. Wilmington, MA
5-0571	Flow Laboratories, Inc., Dublin, VI
5-0591	Harlan Industries, Cumberland, IN
5-0577	Laboratory Supply Co., Indianapolis, IN
5-0579	Murphy Breeding Labs., Plainfield, IN
5-0578	Simonsen Laboratories, Gilroy, CA
5-0599	Southern Animal Farms, Prattville, AL
5-0582	Spartan Research Animals, Inc., Haslett, MI
5-0597	Taconic Farms, Germantown, NY
	18. Diagnosis and Control of Animal Diseases
6-7083	University of Alabama, Birmingham, AL
5-3778	Litton Bionetics, Inc., Kensington, MD
6-7069	Microbiological Associates, Bethesda, MD
8-7157	University of Missouri, Columbia, MO (RFP to be awarded FY 1978)
	19. Production of Athymic - "Nude" - Mice
6-7079	Harlan Industries, Cumberland, IN
	20. Production of Beagle Hounds
6-0125	Hazleton Laboratories, Vienna, VA
6-0124	Laboratory Research Enterprises, Inc., Kalamazoo, MI
6-0123	Marshall Research Animals, Inc., North Rose, NY
	21. Genetic Monitoring of Animal-Tumor Histocompatibility
7-7101	Health Research, Inc., Buffalo, NY

TABLE 1, CONTINUED

Contracts Monitored by DEB During FY 1978

Contract Number	Institute
	22. Development and Publication of Standards and Guidelines for Laboratory Animal Care and Breeding
5-3850	National Academy of Sciences, Washington, D.C.
5-7013	National Academy of Sciences, Washington, D.C.

Projects	Number of Contracts	FY 1978 Funding
Projects 1-13	25	$11,005,720
Projects 14-22	35	7,916,000
Total	60	$18,921,720

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AUTOMATED INFORMATION SECTION

DRUG EVALUATION BRANCH

The Automated Information Section (AIS) is responsible for the implementation and maintenance of the biological data processing system for the Drug Evaluation Branch (DEB), thereby providing for the automated processing, storage, and retrieval of biological data generated in testing laboratories under contract to the DEB, Developmental Therapeutics Program (DTP). Synthetic and natural products are tested in animals for antitumor activity and in cell cultures at various screening laboratories subsequent to entry into the system. On the basis of these test results, decisions are made concerning the direction of compound acquisition, synthesis, fractionation of active crude natural products, and the recommendation of new agents for development toward clinical trial. The biological data base is capable of being queried by special programs thus providing special summary data for: 1) evaluation of the existing screens; 2) design and evaluation of new test systems; 3) review of antitumor activity by the Drug Evaluation Committee, Operating Committee, and Decision Network Committee; 4) detailed evaluation of individual compounds and drug combinations; 5) reports to be included in Investigational New Drug Applications and their updates; and 6) statistical information required for effective program management.

The AIS is staffed by three professionals, one junior programmer, one computer technician and one secretary. Professional staff serves as Project Officers and has responsibility for two contracts, both with Value Engineering Co., N01-CM6-7086T dealt with the total modification of the Plant Header File. This file modification was completed early in the report period and a significant number of plant names which has been in error were corrected. Included among the modifications were, new input form, new update programs, and new output reports.

Under contract number N01-CM3-3706 many enhancements to the BDPS were initiated, among which were the major portion of the programming for the Biological Update Report (BUR) System, analysis of corrections to be made to the master files, and refinements to the Solid Tumor Report Systems. The BUR System is currently on time, consistant with the established milestones. However, an unexpectedly high number of corrections to the master file were required. The analysis of corrections to be made indicated that approximately one man month of effort would be required to affect the changes. However, at the end of the report period nearly eight man months will have been expended. The need for these resulted from file conversions having taken place over the past 10-12 years. The need for corrections is not a reflection of errors, but rather, instances where existing data could not be converted to the new file format. These corrections will be finished in time for the newly reformatted file to become operational during the next report period.

Instruction 14 "Screening Data Summary Interpretation and Outline of Current Screen" was reissued. This version was a loose-leaf notebook which will facilitate deletions, additions, and revisions because individual pages can be added or removed.

An investigation of the feasibility of interfacing the chemical, biological, and inventory files was a major task undertaken during the period. Members of the AIS and Screening Section of the DEB along with Value Engineering Co. participated. Other participants included members of the Drug Design and Chemistry Section, Drug Synthesis and Chemistry Branch, and Chemical Abstracts Services. The final report was submitted to the Deputy Director, DCT who also was an active participant.

A new method of data comparison was presented to the Anthracycline-Coordination Group. The method included collection, analyzing and listing of analog/parent data to assist in identifying possible candidate analogs for clinical trials.

Several reports relative to the Natural Products Processing System were modified. These included the Quarterly Fractionation Report and a supplier inventory report. Data subsystems for both the Plant Header and Confirmed Plant or Animal Materials Report (CPAM) data were designed and implemented.

The BDPS was expanded to include 43 additional test systems and 18 new tumor systems. Several of the new systems were transplantable models of human solid tumors such as the colon and the ovary. Statistics concerning the processing of data are contained in table 1. Again this year there was a reduction in numbers of random compounds screened and an increase in the number of Tumor Panel tests.

AIS processed 230 queries during the reporting period. Among the more difficult queries were the Monthly Materials of Interest, and a quarterly P388/L1210 report for the Natural Products Branch (NPB). This latter report details the number of fractions, the number of animals and number of marine Natural Products supplied by each supplier to the NPB and the test results of screening against mouse leukemias P388 and L1210. Several other queries being done for NPB are designed to minimize the number of duplicate submissions from selected geographic areas of the world.

A significant number of queries were done for users outside of the DTP; some for user's outside of the NCI. Several were queries of the Natural Products files to determine natural products which has been screened previously. Several others were queries of the Single Drug data base to identify highly active structural classes of compounds as an aid in improving selective compound acquisition. Requests for summary data for drugs which were to have been discussed at the DCT Contractors Meeting (canceled) represented one of the largest sets of query output this year.

There is a standing "Quantity Not Sufficient" (QNS) query run biweekly for the IITRI Screening Services contract, NO1-CM-43755, to aid in the scheduling of retests of active compounds. The QNS report lists those materials which need to be acquired and informs staff when they are available. Prior to every Operating Committee meeting a special query and report is run for the Screening Section. Manually generated data extracted from these reports are then input to the Operating Committee Report System and/or the Compounds of Interest File.

Recently, a major programming effort was devoted to development of a system for reporting precise data on animal distribution and costs for the Mammalian

Genetics and Animal Production Section (MG&APS). This will allow DEB to closely monitor animal shipments and costs to DCT contractors, grantees, VRB, V.A. and non DCT users. This report system will permit precise monitoring of animal costs and the full implementation of priorities for animal usage. This report system is expected to be fully operational by the beginning of calendar year 1979.

A new method was developed to transfer Schedule Dependency data directly into a Master File maintained by AIS. This has eliminated the necessity of retrieving data from the Biological Data File retained at DCRT when schedule dependency reports are required and has enhanced the efficiency of producing these reports.

Because of the imminent implementation of the BUR System, the AIS is rewriting all programs for data summarization; 1) Schedule Dependency Subsystems, and its associated master file; 2) all of the query and reporting programs; 3) the monthly and quarterly reports for the NPB report and; 4) Screening Section report for the Operating Committee file.

It is clear from this report that AIS is a necessary and responsive resource for DEB. The relatively great effort devoted to new systems development and reprogramming represents our attempt to meet the Branch's needs regardless of modifications in test systems, experimental protocols, and priorities. The increasing complexity of testing procedures and the need for rapid assimilation and summarization of results from contract testing laboratories throughout the world has progressively increased the need to use automatic data processing systems. At the same time the dynamic nature of the drug testing program requires that the data processing systems be maximally flexible and amenable to change. In addition, in its effort to maximize efficiency in the use of funds testing capacity, and animal resources, DEB has relied heavily on AIS to provide accurate and current utilization reports. Thus, AIS occupies a focal position in the DEB organization and plans to continue its responsivness to program needs.

DCT PROGRAM STATISTICS

	4-1-77 to 3-30-78	1977 1st Quarter	Calendar Year 1976	Calendar Year 1975
1. Materials Added to the Master Files*				
Synthetic Compounds	14,260	6,173	21,127	26,511
Selected Agents	-------	-------	383	175
Plants	6,535	2,225	6,448	6,393
Fermentation Products	2,851	642	1,876	7,328
Animal Products	324	172	1,365	2,086
Total	23,970	9,212	31,199	36,493
2. Tests Performed+				
L1210 Lymphoid Leukemia	16,195	3,704	38,669	160,089
P388 Lymphocytic Leukemia	158,071	65,340	245,949	125,958
Walker Carcinoma	370	183	648	1,318
B16 Melanoma	11,339	2,487	7,349	8,837
Lewis Lung Carcinoma	9,225	648	3,536	5,847
AK Leukemia	-------	-------	50	462
Other Tumors	25,327	3,128	9,952	3,723
Total *In Vivo* (Excluding Toxicity)	220,527	75,490	306,153	306,234
Toxicity	3,970	745	4,683	5,554
Cell Culture	26,193	13,304	33,734	32,931
Alkaloid	255	245	400	350
Biochemical Assay	-------	-------	24	28
Total Non *In Vivo* (Including Toxicity)	30,418	14,784	38,841	38,863
Total of ALL Tests	250,954	89,784	344,994	345,097

* These figures are derived from the Developmental Therapeutics Program Biological Data Processing System and differ from those generated by the Drug Synthesis and Chemistry Branch which logs in materials when NSC numbers are assigned. A material first enters the data processing system when its receipt is acknowledged by the screener, which may be two to four months later.

+ The marked increase in "Other Tumors" represents in part DCT Animal Tumor Panel testing which is delineated further in the current report of the Screening Section, DEB.

MAMMALIAN GENETICS AND ANIMAL PRODUCTION SECTION

DRUG EVALUATION BRANCH

INTRODUCTION

The primary function of the Mammalian Genetics and Animal Production Section is to provide healthy laboratory animals with properly defined genetic characteristics to various research investigators. Recipients during the last year may be catagorized as follows:

1. DCT Contractors
2. Other NCI Research Contractors (DCCP, DCBD, etc.)
3. NIH Intramural Program
4. Research Grantees
5. Veterans Administration Research Facilities

A secondary function of this section is to coordinate the movement of transplantable experimental animal tumors from tumor bank sources into scientific laboratories.

The program is implemented through 34 contracts with qualified institutions.

5 Primary Genetic Centers

7 Rodent Production Centers

10 Hybrid Production Contracts

3 Beagle Production Contracts

1 Histocompatibility Monitoring Contract

2 Contracts with the National Academy of Sciences

1 Contract for maintenance and operation of the DEB main bank of frozen animal and human tumors.

The Section staffing consists of one senior staff member, one professional associate, one animal contracts assistant, and one clerk-typist.

The various animal supply contracts are structured as indicated:

PRIMARY GENETIC CENTERS

These centers serve as a breeding nucleus for the animal program. In order to insure that maximum quality control is maintained at this level, it is required that any animals received by such centers are derived into a germfree state; i.e., Caesarian sections are performed under isolator conditions. These animals are then retained as foundation colonies in defined flora isolators. These foundation isolators serve as a source of breeders for the large expansion colonies which are housed under barrier conditions.

RODENT PRODUCTION CENTERS

The purpose of these contracts is to accomplish large-scale production of pure strains of inbred mice which will be supplied to fixed-price contractors for hybrid mouse production. The rodent production contract receives breeding stock from the genetic center expansion colonies. These breeders, therefore, represent direct offspring of brother x sister matings. This production also takes place under rigid barrier-controlled conditions.

HYBRID MOUSE PRODUCTION CONTRACTS (FIXED PRICE)

These contractors supply the large volume of first generation hybrid mice for the screening program. Breeding animals for these colonies are received from the rodent production contracts. Animals are propagated under semi-barrier conditions.

BEAGLE PRODUCTION CONTRACTS

These animals are procured on a fixed-price contract basis from a qualifed breeder and are primarily to the toxicology program.

DIAGNOSTIC CONTRACTS

These are service contracts which are utilized to monitor and upgrade the quality of animals moving within the animal production program and to the laboratory. Presently, they consist of one virus diagnostic service, two pathology services, one bacterial diagnostic service (primarily Salmonella and Pseudomonas species), and one isolator monitoring (associated flora) contract.

HISTOCOMPATIBILITY STUDY CONTRACTS

These contractors perform genetic monitoring of inbred mouse and rat strains by means of skin grafts.

NATIONAL ACADEMY OF SCIENCES

A small amount of support via Task Orders 6 and 16 is supplied to the National Academy of Sciences. This support involves development and publication of standards and guidelines of the field of animal care and breeding. In addition, support is extended for holding conferences and meetings pertaining to the field of laboratory animal care.

DISCUSSION:

Progress is being made in our animal health program although results from these efforts have been observed at the receiving laboratory level only to a limited degree. Genetic production centers which did not perform satisfactorily for our program, have been phased out. Primary genetic centers have been "closed" to the extent that no animals have entered except through caesarian derivation for about 2 1/2 years. At the three older centers, foundation colonies have been established by brother x sister matings within isolators. Larger expansion colonies have also been established at these centers from offspring of the associated flora maintained isolator colonies. These animals are maintained under strict barrier conditions. Super-clean animals have now became available on a limited basis from these areas and are being supplied to selected rodent production centers. The two newer primary genetic centers have been in existence for less than one year. We estimate that an additional six months will be needed before significant numbers of super-clean animals will be available for distribution to the rodent production centers. However, the newer centers have made satisfactory progress and we can now make projections for future availability of a larger supply of super-clean animals for distribution to rodent production centers.

Rodent Production Center contracts are presently being competed and with one exception awards will be made only to those facilities which have satisfactory barrier conditions for maintaining and expanding production of the super-clean animals that are needed for cancer research. One colony will of necessity be maintained as modified conventional because of the need for a limited number of virus contaminated animals, i.e., (C3H/He+, CD8F1, etc.). Progress in the clean-up of animal production facilities can be summarized presently as follows:

Status FY 75	TYPE OF CONTRACT	Status FY 78
4 (1 clean)	Primary Genetic Center	5 (5 clean)
4 (0 clean)	Genetic Production Center	0
5 (0 clean)	Rodent Production Center	7 (3 clean)
10 (0 clean)	Hybrid Production Center	10 (1 clean)

Hybrid production contracts are scheduled for recompetition with final awards to be made February 1, 1979. Presently, with one exception, all facilities are modified-conventional and will not be satisfactory for producing the super-clean animals that are needed for cancer research. We feel very strongly that awards should not be made to facilities that do not offer barrier conditions and we expect that superior facilities will receive preference and that most if not all awards will be made to these facilities. However, we estimate that the upgrading of these facilities will require an additional outlay of 10¢ per animal or $300,000 per year. We feel very strongly that money should be made available for the upgrading of the hybrid production facilities since this represents the last (and comparatively inexpensive) step in the overall attempt to provide the investigator with animals wherein disease problems will no longer represent potential variables in experimental analyses.

Escalating costs during recent years and budget limitations have impelled DEB to initiate steps to tighten control of animal distribution. MG&APS has been collaborating intensively with the Automated Information Section, DEB to develop a computerized system to account for the distribution of animals and their cost. Principal recipients have been these listed in the first paragraph of this report. The new automated accounting system should be fully operational by the beginning of calendar year 1979. However, preliminary studies have indicated that a substantial number of animals have been distributed gratis to collaborators of DCT personnel and to grantees. Last year steps were taken to arrange for reimbursements for animals distributed to NCI Divisions other than DCT and to the Veterans Administration Laboratories. Currently, steps are being taken to determine how the animal requirements of grantees might be supported. In the meantime, it will no longer be possible to supply animals to grantees and collaborators as a matter of universal policy, and considerable discretion will be required.

SCREENING SECTION

DRUG EVALUATION BRANCH

Principal objectives of the Screening Section are the selection and recommendation of new drugs as candidates for development to clinical trial against cancer and the investigation of means for increasing the therapeutic usefulness of known antitumor drugs. Objectives are attained by: (a) pre-screening a large number of synthetic and natural products *in vivo* against mouse leukemia P388, or to a limited degree, *in vitro*; (b) selecting pure compounds for testing against a spectrum of animal tumors and human tumor xenografts; (c) conduct of biological assays for directing fractionation of natural products and isolation of active components; (d) in-depth evaluation of materials which emerge as active from antitumor screens with emphasis on the influence of factors such as route and treatment schedule that affect therapeutic efficacy; (e) analysis, evaluation, and presentation of pertinent experimental results to Division of Cancer Treatment (DCT) Staff; and (f) methodological studies leading to development of improved assays for drug evaluation. Program is implemented through establishment and coordination of work conducted by contractual arrangement with qualified institutions, and through collaborations with DCT intramural laboratories. Activity against experimental tumors *in vivo* is the principal basis for recommending candidate drugs for development to clinical trial and for studying optimal conditions for drug usage. *In vitro* activity against mammalian tumor cells or microorganisms provides a means of recognizing biological activity among synthetic agents submitted in insufficient quantity for *in vivo* screening, for following fractionation and isolation of active components of crude natural products with correlative *in vivo* activity, and for providing information relative to mechanisms of drug actions.

I. Staff Functions

During this report period the staffing of the Screening Section has included five senior professionals, one junior professional, and five technical and clerical personnel. Principal investigators are: B. J. Abbott (head); R. I. Geran; N. H. Greenberg; M. M. Macdonald; and N. R. Melnick.

Staff serve as Project Officers for nine contracts devoted predominantly to *in vivo* screening: Arthur D. Little, Inc. (N01-CM5-7006), Battelle Columbus Laboratories (N01-CM5-7005 and N01-CM6-7099), Hazleton Laboratories (N01-CM5-7007), IIT Research Institute (N01-CM5-7004), Institute Jules Bordet (N01-CM5-7040), "Mario Negri" Institute for Pharmacological Research (NO1-CM3-3720), Mason Research Institute (NO1-CM[illegible]-[illegible]003[illegible]) and Raltech Scientific Services, Inc., formerly WARF Institute, Inc. (NO1-[illegible]-3770). Although the number of contractors conducting predominantly *in vivo* screening increased at the time of the last competition, the average test capacity per contract, as well as total *in vivo* test capacity, has been reduced due to cost inflation and program changes. Staff also serve as Project Officers for five *in vitro* screening contracts: Arthur D. Little, Inc. (NO1-CM6-7078), IIT Research Institute (NO1-CM6-3832), Southwest Foundation for Research and Education (NO1-CM6-7075), University of Miami (NO1-CM6-7077), and University of Wisconsin (NO1-CM6-7076). The *in vitro* screening contracts will be recompeted during this year. A Screening Services Contract with IIT Research Institute (NO1-CM4-3755), to be recompeted in 1978, assists staff in the management of screening data which has become increasingly complex as protocols have been modified and expanded in recent years.

The Screening Section collaborates with the Chief, Drug Evaluation Branch (DEB); Chief, Natural Products Branch (NPD); Assistant Director for International Treatment Research (DCT); and the Associate Director, Developmental Therapeutics Program (DTP) to monitor screening, detailed drug evaluation and methodology performed under contracts with Catholic Medical Center (NO1-CM6-7081), Southern Research Institute (NO1-CM4-3756), Arthur D. Little, Inc. (NO1-CM3-3727 and NO1-CM5-3765), Upjohn Company (NO1-CM7-7100), Michigan Department of Health (NO1-CM3-3702), Arizona State University (NO1-CM6-7048), W. R. Grace (NO1-CM6-7074), Purdue University (NO1-CM6-7091), Institute Jules Bordet (NO1-CM5-3840), Japanese Foundation for Cancer Research (NO1-CM2-2054), The Institute for Cancer Research, England (NO1-CM4-3736), and Research Triangle Institute (NO1-CM9-2019).

By reviewing biological effects of natural product fractions, the Screening Section establishes directions of effort under additional DTP contracts for natural product fractionation and isolation. Staff monitor the work of contract laboratories to insure that tasks are carried out as scheduled with a high degree of scientific and technical proficiency and to guarantee that they remain directed to the overall goals of DTP, DCT, NCI and the National Cancer Plan. Staff select assays for uncovering new active drugs, design experimental protocols for contractor use, review results, recommend candidate drugs for development toward clinical trial, and prepare data for publication and review by the Drug Evaluation Committee, Operating Committee, Laboratory of Toxicology, Clinical Trials Area, and for DCT Decision Network Committee.

II. Significant Accomplishments

A. Determination of Antitumor Activity of New Agents

Primary screening *in vivo* consists of the initial testing of synthetic and natural products for activity against transplantable mouse tumors. Screening protocols are designed to uncover a modest number of materials which are then subjected to progressively exacting requirements to select those with the greatest potential.

Transplantable mouse leukemia P388 remained the initial in vivo screen for most materials submitted for testing. The reasons for selecting P388 as the initial screening model or "pre-screen" were reported previously. Materials which are active in our pre-screen, materials which have demonstrated interesting activity outside our program (e.g., antitumor activity in other screens or pertinent biochemical or biological activities) and some of special interest because of the rationale for synthesis or their structure are tested against a panel of eight experimental tumor models. This secondary screening panel includes mouse colon, breast, and lung tumors and human tumor xenografts of these types, a mouse melanoma, and mouse leukemia L1210. Other tumors such as brain, renal, ovarian or bladder may be used on a selective basis when appropriate. Tumor systems have been phased into the panel as permitted by the completion of developmental work, the availability of laboratory testing capacity and the mouse host, and in the case of human tumor xenografts, particularly the availability of athymic (nude) mice. The tumor panel now includes the following test systems and capacity:

B16 melanocarcinoma: Capacity to test 1000 compounds per year was available by February 1976 and is continuing at that level.

$CD8F_1$ mammary tumor: Capacity was limited to 75 compounds per year until April of 1977 when additional animals began to be available. Test capacity increased to 750 compounds per year by January 1978. Some of this test capacity continues to be utilized for the evaluation of experimental protocols.

Colon 38 adenocarcinoma: Test capacity in March 1976 was 250 compounds per year when working protocols were developed and increased to 1000 compounds per year by August 1977.

L1210 lymphoid leukemia: Test capacity is 1000 compounds per year.

Lewis lung carcinoma: Test capacity is 1000 compounds per year.

Xenografts - colon, mammary and lung: At the inception of the tumor panel concept, there was no capacity for testing in any of these systems. The first animal production contract for athymic mice was signed May 1976. The contract for screening was signed in September 1976, when 50 animals per week were available. Animal disease problems were encountered at both the screening laboratory and at the production colonies during 1977. Xenograft test capacity in December 1977 was approximately 250 compounds per year and it is expected to be about 375 by July 1978.

The effort required to adequately test a compound in the panel of secondary screens is much greater than that required for follow-up testing of active materials in the past. Therefore, the number of materials tested in this panel is limited and the input of new materials to the pre-screen has been reduced. Since the initiation of the tumor panel, 882 compounds have been recommended for testing in these systems. Of these 561 (329 during this reporting period) were selected on the basis of activity in the P388 pre-screen. The remainder by-passed the P388 pre-screen and were tested against the tumor panel because of other demonstrated biological or biochemical activity or as part of "analog" comparison studies. Materials regarded as potential candidates for clinical trial are examined for route and schedule dependency. Analogs of compounds already in development are compared with the parent compound in test systems selected on the basis of the biological activity of the parent. Experimental and final clinical formulations are tested for activity whenever appropriate. Crude natural products continue to be tested both in vitro (KB) and against the P388 in mice. Where activity is observed in both, the less expensive cell culture assay is used for bioassay to follow activity during the process of fractionation.

Tables 1-3 show the number of screening tests initiated and computer processed during the period April 1, 1977 through March 31, 1978. An in vivo test is defined as a treated group of from six to ten animals. An in vitro test consists of a concentration - response assay from which the cytotoxic ED50 is extrapolated. Of 235,267 total tests for this 12-month period, 207,770 were in vivo (Table 2) as compared with 234,487 tests reported for the same period last year. From April 1, 1977 through March 31, 1978, 27,497 in vitro tests were conducted (Table 3) as compared with 31,080 for the same period last year. Tables 1-3 list the number of tests conducted with "selected agents" (SAC) which are chosen for further study because of activity in initial screening or other biological activity of interest, other synthetic compounds (Regular), structural congeners of known active compounds (Analogs), crude plant extracts, crude fermentation beers, crude animal extracts, and natural product fractions. These numbers include tests processed in our main computerized single drug data file. They exclude special testing such as combination chemotherapy and a separate solid tumor data file, and testing in new systems under current development for which results are not computer processed. For example, there were 10,817 tests processed during this same period on the combination chemotherapy file, which are not included in these tables. Although the total number of in vivo tests (Table 2) decreased from the number reported for the comparable 12-month period last year, the percentage of the total in vivo tests carried out on regular selected (SAC) agents increased from 13% to 28%. This is a direct result of implementation of the plan to test materials active in a pre-screen in a broader spectrum of tumor test systems in which the cost per test is substantially greater than for the P388 prescreen.

Table 4 shows, by type, the number of materials first tested in any test system during the period April 1, 1977 through March 31, 1978. The total, 23,934, is the number of materials for which testing was initiated during this report period and processed by April 14, 1978. This number reflects the reduction (approximately 30%) of the number of new materials tested in the comparable period last year. Table 4 also gives an analysis of the average lag time between the date a test is initiated and the date that test results are computer processed. This lag time is greatest for selected agents and markedly lower for other synthetics or natural products. In general, initial screening is completed in a relatively short time. As materials are determined to be active or require specialized testing, they are moved to the SAC file. The longer lag time for the latter is a reflection of both activity and more sophisticated test systems requiring more time for completion.

Table 5 shows, by test system, the number of tests carried out during this report period in the tumor models in the DCT panel of tumors. These numbers include the testing of compounds recommended for the complete panel as well as the testing of compounds recommended for a limited spectrum of panel tumors, e.g., analogs or compounds of special interest tested in a few selected panel tumors and the P388 pre-screen. The Colon 26 and Ependymoblastoma tumor models are not in routine use in the panel but are included in Table 5 because the number of tests were significant, and they are examples of the ancillary test systems utilized by the program. Table 5 also shows that testing level in the xenograft systems is still low relative to animal tumor testing because of the lack of a precedent for xenograft screening when the project was begun and the lack of mice and facilities early in its development. Initially, available xenograft testing capacity was mainly utilized to establish protocols and to test known agents; such as, clinically effective drugs and those of special interest to the DCT program. Currently, only two laboratories have the required facilities necessary for working with the human tumor xenografts and nude mice. However, it is anticipated that shortly two additional laboratories will convert some of their existing test capacity to xenograft testing.

Compounds recommended for panel testing are: a) selected on the basis of pre-screen activity by the Data Review Subcommittee, b) priority analogs selected by coordinating committees, c) compounds currently in development (Operating Committee), and d) compounds of special interest to DCT program staff.

B. Selection of Candidate Drugs for Clinical Trial

During this report period, 1,395 compounds were added to the Selected Agents (SAC) list because of demonstrated confirmed *in vivo* or *in vitro* activity for the first time (Table 6). 1,327 (95%) of these new actives were found in *in vivo* tumor systems, the majority, 91%, showing initial activity in the P388 screen. Sixty eight compounds were added to the SAC list because of cell culture activity.

The Data Review Subcommittee (DRS) of the Drug Evaluation Committee (DEC) reviewed screening results for 2,289 compounds and referred 13 to the DEC. The DEC reviewed a total of 98 compounds for activity worthy of presentation to the DCT Decision Network Committee. Thirteen new active agents passed DCT Decision Network point 2A (DN2A) (Table 7).

C. Detailed Evaluation of New and Old Drugs of Particular Interest

Since the inception of the NCI screening program over twenty years ago approximately 577,000 materials have been screened as possible anticancer agents. Approximately 282,000 of these have been natural products and 295,000 have been synthetic agents. During this report period the new system of pre-selection of synthetic materials has increased to 10% the rate of active materials selected by the screen. From among all of the materials tested, 8,729 (1.3%) are currently considered to be of particular interest and the file of these Selected Agent Compounds (SAC), maintained by the section, contains comprehensive records of experimental results for active drugs and other materials of special interest. Approximately one-fifth of these are being actively pursued on their own merit and the others are included in comparison studies with related drugs. New data received from this program, other DTP contract or intramural laboratories, non-NCI sponsored drug evaluation programs, and the literature is reviewed and when warranted these materials are presented to appropriate NCI committees for review.

The Screening Section is responsible for the detailed evaluation of drugs which passed DN2A including an evaluation of possible schedule dependency, toxicity in non-tumor bearing rodents, activity against the DCT panel of tumors, biological quality control of experimental clinical formulations, and evaluation of the extent to which modifications in drug usage might increase therapeutic efficacy, including combination chemotherapy and combined modalities.

In collaboration with the Office of the Chief, DEB, results are reviewed and summarized for Decision Points subsequent to DN2A, for inclusion in clinical brochures and IND applications, and for presentation to clinical working groups.

1. Schedule Dependency Studies. Schedule dependency studies were initiated during this period in the following murine models: three in the B16 melanocarcinoma, one in Lieberman plasma cell No. 1, one in M5076 ovarian carcinoma, one in L1210 lymphoid leukemia, and one in P388 lymphocytic leukemia.

2. Combination Chemotherapy Studies. An offline computer file of animal data is maintained for those therapy combinations that have been tested in rodent models (some in several models, in several experiments, and at several laboratories). An average of about ten new unique drug combinations are being tested per month. At the end of 1977 data for 1,078 unique drug combinations were on record. An attempt is made to publicize those combination treatments that appear to be most promising.

3. Biological Quality Control of Experimental Clinical Formulations. Comparative testing of bulk drug and various experimental clinical formulations was conducted for six agents in development toward clinical trial. Such testing, which may require the evaluation of a number of experimental forms for each drug, is necessary to insure against loss or reduction of antitumor efficacy during the process of formulation.

4. Screening of Structural Congeners of Active Drugs. In vivo testing of congeners was conducted at Arthur D. Little, Inc. (N01-CM5-3765) using animal-tumor models selected on the basis of the qualitative and quantitative activity of the parent compound. While the main objective of this project is to develop congeners with potential clinical superiority, it also provides information to aid in the selection of approaches to new synthesis. Materials are tested within approximately one week of receipt in the laboratory and results are sent directly to the supplier of the material.

   This project entails (a) identification by DCT of drug classes of special interest, in particular the major classes of clinically active drugs; (b) acquisition of new structural congeners and other rationally-designed drugs; and (c) biological comparative testing of congeners to determine potential superiority over the parent drug or uniqueness of action.

D. Methodology (Research and Development)

This past year the emphasis in this area was devoted to the finalization of protocols for tumors in the "Tumor Panel" and modifications of existing "Non-tumor Panel" protocols. Because of this commitment, research and development at laboratories on individual projects was decreased and replaced by coordinated research projects involving multi-laboratory collaborations. Major projects are listed below:

1. A host comparison study was initiated last year to compare the efficacy of several known materials in both the C57Bl and B6C3Fl mice with the aim of substituting the B6C3Fl mouse for the C57Bl mouse in the ependymoblastoma tumor model. During this past year this study was completed. Based upon statistical analysis, the B6C3Fl mouse has been substituted for the C57Bl mouse.

2. The study to determine the feasibility of substituting the B6C3Fl mouse for the BDFl mouse as host in the B16 melanoma tumor model was completed. Based upon the statistical analysis of this study, this substitution was made and the initial T/C value for preliminary efficacy was lowered from a value of 140 to 125.

3. During this past year, several laboratories conducted coordinated research utilizing the Colon 26 and Colon 38 tumors. This testing was statistically analyzed and was in addition to the Colon 26 Tumor Panel testing. As a result of this study, a finalized protocol was issued for both tumors which differed from the tentative protocols. Changes in the Colon 26 protocol are: (a) in tumor brei preparation, (b) level of inoculum, (c) treatment regimen, (d) weight range of host animals, (e) final weight and toxicity day, and (f) final evaluation day. Currently, a study is in progress to finalize the acceptable control median survival time limits.

   The changes in the finalized Colon 38 tumor protocol were not as extensive as that for the Colon 26 protocol. The treatment regimen and the weight range of the host animals were the only changes. Since the Colon 38 tumor is a solid tumor evaluated by tumor inhibition, it may be affected by caloric restriction. A study is planned to determine the effect of caloric restriction on tumor growth.

   Currently several studies are in progress or proposed and they are listed below. The results of these studies will be statistically analyzed prior to the issuance of new or modified protocols.

   a) $CD8F_1$ Mammary Tumor: IIT Research Institute, Southern Research Institute.

      It was necessary to expand this model to two additional laboratories. The resulting increase in available test data uncovered problems inherent to model as it is currently used. Because the model involves the use of first generation transplants for testing, the tumor inoculum used for each experiment is derived from a different spontaneous tumor. Under these circumstances, variation in tumor growth rate and response to therapy from experiment to experiment occurs and is not unexpected. Pooling of donor tumors has not solved this problem. As part of an effort to standardize this system, studies are in progress to define a more reliable treatment regimen for general screening and to determine the effect of caloric restriction on tumor growth. The results of these studies will be statistically analyzed and a finalized protocol issued. In addition, we are exploring the possibility of using novel statistical methods to deal with the relatively wide variation in test results since such variations may be a reality for this model rather than an experimental artifact.

b) Lewis Lung Carcinoma: Arthur D. Little, Inc., Battelle Columbus, Hazleton Laboratories, IIT Research Institute, Raltech Scientific Services, Inc., Southern Research Institute.

A study is nearly complete to determine the feasibility of substituting the B6C3F1 mouse for the BDF1 mouse as host animal. All testing has been completed and a statistical analysis is being conducted.

c) Xenografts: Battelle Columbus, Stehlin Foundation for Cancer Research.

Because of the limitation on the number of xenograft models that can be used in the tumor panel, the problem of selection from a number representing each human tumor type exists. At the Mason Research Institute, a technique for growing the human tumors in the renal capsule of nude mice has been perfected. Although this rapid and relatively inexpensive system is not intended to replace the current xenograft tumor models, it is conceivable that it could be used as a pre-screen against a relatively large number of human tumors. "Active" compounds could then be tested for therapeutic efficacy against the established subcutaneous tumor that showed greatest sensitivity in the kidney capsule. A study of correlations between activity in the kidney capsule and the usual subcutaneous tumor models is being conducted.

d) Colon 38: Battelle Columbus, Hazleton Laboratories, IIT Research Institute, Mason Research Institute, Raltech Scientific Services, Inc., Southern Research Institute.

Although a finalized protocol for testing in the Colon 38 tumor system was issued this past year, a study was initiated at several laboratories to determine if more accurate data would be obtained if the tumors were excised and weighed rather than estimating the tumor weight from caliper measurements.

e) M5076 Ovarian Tumor: Arthur D. Little, Inc., Mason Research Institute, Southern Research Institute.

In order to further evaluate this ovarian tumor prior to its possible inclusion in the available tumor listing, a study has been initiated at several laboratories to assess the effect of caloric restriction and the efficacy of several known antitumor drugs.

f) Mammary 16C Tumor: IIT Research Institute, Raltech Scientific Services, Inc., Southern Research Institute.

A tentative protocol for testing against mammary adenocarcinoma 16C, derived from a spontaneous C_3H mouse tumor, has been established. The time required to test an agent in the 16C model is approximately half of that required for testing against the $CD8F_1$ mammary carcinoma. In addition, the test host mouse for 16C is the B6C3F1 strain which is currently the test host for B16 melanoma and the ependymoblastoma. Preliminary test data does not disclose the variation in tumor growth that exists with the $CD8F_1$ tumor. Studies of drug efficacy and caloric restriction are scheduled. It will be interesting to know whether the current panel mouse mammary tumor, $CD8F_1$, a relatively costly and variable model, responds to therapy differently from the B6C3F1 model.

Several contract laboratories conducted independent developmental studies in addition to the program directed studies listed above. Selected examples are given below.

a) Southern Research Institute:

Kinetic studies of the CD8F1 mammary tumor disclosed: (1) that the growth rate of the first transplant generation was not related to the growth rate of the host tumor, (2) the number of viable tumor cells implanted influenced the ultimate size of the tumor on a given day, but other factors may influence tumor size, and (3) there is a direct relationship between the number of viable cells implanted and the number of no-takes.

b) Battelle Columbus:

Based upon testing experience, the Mammary, Lung, and Colon zenograft protocols were revised. The following agents demonstrated some degree of efficacy. Mammary: AT-125, Azotomycin, AZQ, Chlorozotocin, D-O-Norleucine, PALA, Pentamethylmelamine, Piperazinedione, 6-Thioguanine, Trimethyltrimethylolmelamine, Procarbazine. Lung: AT-125, Azotomycin, D-O-Norleucine. CX-2 Colon: Azotomycin, Lymphosarcin, D-O-Norleucine, Pentamethylmelamine.

c) Arthur D. Little, Inc.:

Studies of the relationship of route of administration (IP vs IV) to efficacy against IP implanted P388 showed that Adriamycin, Vincristine and Melphalan were superior when given IP rather than when given IV. With Methotrexate, 6-Mercaptopurine, 5-Fluorouracil, Vincristine, Cyclophosphamide, and BCNU the route of administration did not influence efficacy substantially.

III. Related Projects

The need for uniformity among contract laboratories in a large screening program requires persistent surveillance. It is the responsibility of the Screening Section to assure uniform biological characteristics of tumor lines as well as host animals. To achieve this, frozen tumor banks are maintained by several contract laboratories for primary lines in addition to the major contract for dispersal of new tumor lines to screeners on a periodic basis.

The requirement for host animals in good health and in large numbers necessitates close liaison with the Mammalian Genetics and Animal Production Section, DEB, whose contract laboratories perform studies related to the diagnosis and control of animal disease and monitor the quality of the animals utilized in the program. During this report period, when lack of funding has made it difficult for animal production to meet screening needs, it has been necessary to hold frequent meetings and assign animal shipments to screeners in order to insure maximum animal utilization and minimal backlogs in tumor panel systems.

The Screening Section, as the major user of the biological data processing system, continues to work closely with the Automated Information Section, DEB, by advising and assisting in the planning of modifications and the monitoring of data necessary to an ongoing data processing system. This year, with major enhancements being programmed for this system, increased staff time has had to be committed to this area in both planning and data review.

IV. Proposed Course of Projects

The Screening Section will continue to supervise the testing and evaluation of data for all materials screened in the DTP program. This includes the continuous evaluation of the primary screen and its criteria, logistics and tumor models. Research and methodology will be carried out under contract for related projects of interest to DCT, NCI. Such projects are directed toward devising new or improved antitumor assays, evaluation of new test systems for their potential as screening tools, and the development of standardized methodology to be used by the participating contract laboratories.

TABLE 1

NUMBER OF IN VIVO AND IN VITRO SCREENING TESTS CONDUCTED
FROM APRIL 1, 1977, THROUGH MARCH 31, 1978*

	Selected Agents Compounds (SAC)		Other Synthetics				Natural Products		
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	Total	
1977									
Second Quarter	16,172	2,056	24,356	2,613	13,165	1,511	3,247	17,923	(Crude)
					3,153	2,314	3,317	8,784	(Fraction)
					16,318	3,825	6,564	26,707	(Both)
Third Quarter	17,049	2,887	20,392	1,727	13,115	579	3,618	17,312	(Crude)
					2,612	1,307	3,077	6,996	(Fraction)
					15,727	1,886	6,695	24,308	(Both)
Fourth Quarter	16,358	2,498	14,136	1,329	9,338	172	3,262	12,772	(Crude)
					2,870	1,424	3,572	7,866	(Fraction)
					12,208	1,596	6,834	20,638	(Both)
1978									
First Quarter	10,115	1,374	9,929	919	10,312	42	3,649	14,003	(Crude)
					2,562	1,349	1,790	5,701	(Fraction)
					12,874	1,391	5,439	19,704	(Both)
Total	59,694	8,815	68,813	6,588	57,127	8,698	25,532	91,357	

	Second Quarter 1977	Third Quarter 1977	Fourth Quarter 1977	First Quarter 1978	Four-Quarter Period
Total All Tests	71,904	66,363	54,959	42,041	235,267

*Includes tests begun no earlier than April 1, 1977, and reported to the Automated Data Processing (ADP) Contractor by April 14, 1978.

TABLE 2

NUMBER OF IN VIVO SCREENING TESTS CONDUCTED
FROM APRIL 1, 1977, THROUGH MARCH 31, 1978*

	Selected Agents Compounds (SAC)		Other Synthetics		Natural Products			
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	Total
1977								
Second Quarter	15,865	2,056	24,177	2,613	9,589 1,898 11,487	1,122 607 1,729	2,614 3,065 5,679	13,325 (Crude) 5,570 (Fraction) 18,895 (Both)
Third Quarter	16,858	2,887	20,275	1,727	9,477 1,369 10,846	473 454 927	2,715 2,716 5,431	12,665 (Crude) 4,539 (Fraction) 17,204 (Both)
Fourth Quarter	16,219	2,498	13,962	1,329	6,745 1,934 8,679	95 609 704	2,223 3,303 5,526	9,063 (Crude) 5,846 (Fraction) 14,909 (Both)
1978								
First Quarter	10,026	1,374	9,741	919	8,536 1,283 9,819	29 397 426	2,434 1,557 3,991	10,999 (Crude) 3,237 (Fraction) 14,236 (Both)
Total	58,968	8,815	68,155	6,588	40,831	3,786	20,627	65,244

	Second Quarter 1977	Third Quarter 1977	Fourth Quarter 1977	First Quarter 1978	Four-Quarter Period
Total All In Vivo Tests	63,606	58,951	48,917	36,296	207,770

*Includes tests begun no earlier than April 1, 1977, and reported to the Automated Data Processing (ADP) Contractor by April 14, 1978.

TABLE 3

NUMBER OF IN VITRO SCREENING TESTS CONDUCTED FROM APRIL 1, 1977, THROUGH MARCH 31, 1978*

	Selected Agents Compounds (SAC)		Other Synthetics		Natural Products			
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	Total
1977								
Second Quarter	307		179		3,576	389	633	4,598 (Crude)
					1,255	1,707	252	3,214 (Fraction)
					4,831	2,096	885	7,812 (Both)
Third Quarter	191		117		3,638	106	903	4,647 (Crude)
					1,243	853	361	2,457 (Fraction)
					4,881	959	1,264	7,104 (Both)
Fourth Quarter	139		174		2,593	77	1,039	3,709 (Crude)
					936	815	269	2,020 (Fraction)
					3,529	892	1,308	5,729 (Both)
1978								
First Quarter	89		188		1,776	13	1,215	3,004 (Crude)
					1,279	952	233	2,464 (Fraction)
					3,055	965	1,448	5,468 (Both)
Total	726		658		16,296	4,912	4,905	26,113

	Second Quarter 1977	Third Quarter 1977	Fourth Quarter 1977	First Quarter 1978	Four-Quarter Period
Total All In Vitro Tests	8,298	7,412	6,042	5,745	27,497

*Includes tests begun no earlier than April 1, 1977, and reported to the Automated Data Processing (ADP) Contractor by April 14, 1978.

TABLE 4

SUMMARY OF MATERIALS FIRST TESTED IN ANY SYSTEM
FROM APRIL 1, 1977, THROUGH MARCH 31, 1978*

	Selected Agents Compounds (SAC)			Synthetics			Natural Products				
	Synthetic	Analog	Total	Synthetic	Analog	Total	Plants	Animals	Fermentations	Total	Total
1977											
Second Quarter	205	26	231	4,867	424	5,291	1,594	203	735	2,532	8,054
Third Quarter	187	43	230	4,617	241	4,858	1,973	34	647	2,654	7,742
Fourth Quarter	39	32	71	2,337	183	2,520	1,467	7	483	1,957	4,548
1978											
First Quarter	3	0	3	1,465	105	1,570	1,222	5	790	2,017	3,590
Total	434	101	535	13,286	953	14,239	6,256	249	2,655	9,160	23,934

Analysis of lag time between the date the tests were initiated at the laboratory (date on) and the date the test results are processed reveals that the following percentages of the data have been processed in the second, third, and fourth months after the date-on month:

	SAC		Synthetics		Natural Products	
Month Beyond Date On	Average	Range	Average	Range	Average	Range
Second	40%	29%-49%	66%	34%-86%	82%	65%-93%
Third	77%	62%-87%	91%	79%-97%	98%	89%-99%
Fourth	93%	90%-97%	98%	95%-99%	99%	98%-99%

*Includes compounds for which test results were processed by April 14, 1978.

TABLE 5

NUMBER OF TESTS CONDUCTED AGAINST PANEL TUMOR SYSTEMS FROM APRIL 1, 1977, THROUGH MARCH 31, 1978*

Panel Tumor Systems	Second Quarter 1977	Third Quarter 1977	Fourth Quarter 1977	First Quarter 1978	Total
L1210 Lymphoid Leukemia (LE)	4,237	3,288	3,085	2,624	13,234
B16 Melanocarcinoma (B1)	2,449	2,349	3,236	1,662	9,696
Lewis Lung Carcinoma (LL)	2,268	2,750	2,012	1,323	8,353
$CD8F_1$ Mammary Tumor (CD)	1,124	1,287	1,587	1,271	5,269
Colon 26 (C6)	1,308	1,657	2,474	661	6,100
Colon 38 (C8)	833	920	1,666	1,554	4,973
Ependymoblastoma (EM)	978	830	759	1,028	3,595
CX-1 Colon Xenograft (C2)	16	62	5	0	83
CX-2 Colon Xenograft (C4)	36	91	34	0	161
LX-1 Lung Xenograft (LK)	42	48	82	10	182
MX-1 Breast Xenograft (MB)	24	83	14	0	121
Total All Panel Tumor Systems	13,315	13,365	14,954	10,133	51,767

*Includes tests begun no earlier than April 1, 1977, and reported to the Automated Data Processing (ADP) Contractor by April 14, 1978.

TABLE 6

New Confirmed Active Compounds added to the Selected Agents Listing for the last three Quarters of 1977, and the first Quarter of 1978.

Total Number of Materials	Number of Materials by Tumor System
1,327 *in vivo*	1,202 P388 Leukemia
	73 L1210 Leukemia
	2 W256 - IM
	19 W256 - IP
	4 B16 Melanoma
	13 Lewis Lung
	1 Ependymoblastoma
	6 LePage Gardner Lymphosarcoma
	7 Madison Lung
68 *in vitro*	68 KB cell culture

TABLE 7

Status of Active Compounds

For FY 1978

New Confirmed Actives	1,395
Compounds Reviewed	
DRS	2,289
DEC	98
Total	2,387
Passed DN2	13
Route and Schedule Dependency Studies	
Scheduled	7
Reviewed by Screening Committee	17

TABLE 8

COMPOUNDS ASSIGNED TO DN-2 FOR THE FIRST TIME 4/1/77 THROUGH 3/31/78

NSC#	NAME	DATE OF ASSIGNMENT	BASIS FOR ASSIGNMENT (ACTIVITY)
* 21548	Thymidine	77-04-18	Activity of high dose thymidine vs human melanoma xenografts
125973	Taxol; Tax-11-en-9-one,5β,20-epoxy-1,2α,4,7β,10β,13α-hexahydroxy-,4,10-diacetate, 2-benzoate 13-(α-phenylhippurate)	77-04-18	B16 melanoma
143108	Dibutyryl Cyclic AMP; Butyramide,N(9-β-D-ribofuranosyl-9H-purin-6-yl)-,2'-butyrate cyclic 3',5'-(hydrogen phosphate)	78-03-29	Biochemical Rationale-Activity against estrogen dependent DMBA-induced rat mammary tumor and MTW9 transplantable rat mammary tumor.
196473	Anthracenedione; 9,10-anthracenedione,1,4-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-	77-04-18	B16 melanoma
201628	Carbonic dihyrazide,bis[(3,5-dibromo-2-hydroxyphenyl)methylene]-	77-10-08	B16 melanoma
234714	Aphidicolin; 8,11a-methano-11aH-cyclohepta[a]napthalene-4,9-dimethanol,tetradeca=hydro-3,9-dihydroxy-4,11b-dimethyl-,[3R-(3α,4α,4aα,6aβ,8β,9β,11aβ,11bβ)]-	77-11-30	Colon 26 carcinoma
237020	Largomycin	78-03-29	B16 melanoma

TABLE 8

NSC#	NAME	DATE OF ASSIGNMENT	BASIS FOR ASSIGNMENT (ACTIVITY)
261036	RO-5-9963; 1,2-Propanediol,3-(2-nitro-1*H*-imidazol-1-yl)-	78-03-29	Radiosensitizer
287513	Anthracenedione,diacetate; 9,10-anthracenedione,1,4-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-,diacetate(salt)	77-11-30	Replaces NSC#196473 on basis of greater solubility. B16 melanoma
288411	Indenoquinoline carboxylic acid,sodium salt; 6*H*-Benzo[*b*]carbazole-11-carboxylic acid,sodium salt	78-03-29	B16 melanoma
289642	Macromomycin (without chromophore)	77-11-30	Replaces NSC#170105 as contains none of the chromophore contaminant. B16 melanoma
296961	WR-2721;Ethanethiol,2-[(3-aminopropyl)amino]-,dihydrogen phosphate (ester)	78-03-29	Radioprotector
526417	Quinomycin A (Echinomycin)	78-03-29	B16 melanoma

*Passed Decision Network at the DN-3 level since toxicology has been completed.

SUMMARY REPORT OF THE PHARMACEUTICAL RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1977 - September 30, 1978

The Pharmaceutical Resources Branch, established in July, 1976, is structured to provide comprehensive pharmaceutical services to the various programs of the Division of Cancer Treatment. The primary objectives of the Branch are to supply high quality chemical substances and formulated products for investigative program use. These objectives are accomplished essentially through contract support activities. The major contract areas include: chemical preparation and pilot plant production; analytical services; pharmaceutical research and development; and pharmaceutical manufacturing. The synthesis and distribution of radiolabeled chemicals and drugs are also provided through PRB contract sources. Additionally, the Branch is responsible for storage, distribution and computerized inventory maintenance of all drug products used in the Clinical Programs.

TABLE I

RESOURCE CONTRACTS MONITORED BY
THE PHARMACEUTICAL RESOURCES BRANCH

Chemical Preparation	9
Radiolabel Synthesis	2
Pharmaceutical R & D	1
Pharmaceutical Production	4
Analytical Services	2
Drug Distribution	1
Total Resource Contracts	19

The Branch is also responsible for direct purchase of chemicals and drug products utilizing NIH procurement services.

A formulation research laboratory on the NIH campus is operated and staffed by the Pharmaceutical Resources Branch. This laboratory is assigned research projects of high program interest which present difficult drug delivery problems. Most of the chemical agents developed by the Laboratory of Medicinal Chemistry and Biology are assigned to this laboratory. This arrangement facilitates a scientific exchange between the developer and formulator and a team approach to the ultimate product design.

Branch Staff

The Pharmaceutical Resources Branch is presently staffed with seven senior professionals, two technical and three clerical personnel. In addition, two Visiting Fellow positions are assigned to the Formulation Laboratory. The classification of the senior professionals is as follows: four PHS Commisioned Corps pharmacists; one analytical chemist; one Ph.D. medicinal chemist; and one staff fellow.

The Branch consists of three Sections:

1. Chemical Resources Section - Head, Dr. Robert R. Engle

   The primary functions of the Chemical Resources Section are to provide for resynthesis, large-scale production and procurement services for acquisition of chemical substances. These services are accomplished by the management and supervision of a contract program for (a) resynthesis of small quantities of bulk substances as required for preclinical assessment and (b) pilot plant production of various quantities of bulk substances intended for pharmaceutical manufacture of clinical investigational products. The Section also initiates the acquisition of commercially available chemical substances through NIH procurement contract procedures. This requires the preparation of stringent material specifications to insure that high quality products are obtained.

   Another major function of the Section involves the management of a contract program to prepare and distribute radiolabeled materials. These materials are distributed to authorized investigators for clinical pharmacology and other related studies.

   This Section supervises nine chemical prep lab contracts and two radiolabel synthesis laboratory contracts.

2. Analytical and Product Development Section - Head, Mr. James C. Cradock

   This Section is responsible for the quality assessment of chemicals and formulated drugs used in the Division of Cancer Treatment's drug evaluation program. Analyses are conducted by contract laboratories independent of chemical or formulated drug suppliers. This Section is also responsible for the development of analytical methodology to determine the purity of chemicals, potency of active ingredients in pharmaceutical formulations, stability of formulated products under accelerated and simulated use conditions and identification of impurities and/or degradation products. Other evaluations include determination of partition coefficients for use by other DTP components in structure activity studies and application of analytical techniques to estimation of certain compounds in biological fluids. Data are used for preparation of analytical profiles of bulk drugs, designation of reference standards and preparation of purchase specifications of bulk chemicals.

A second major function regards the development of suitable pharmaceutical dosage forms for clinical trial. Since most preparations are intended for intravenous use, studies are undertaken to assess the solubility and stability in a variety of pharmaceutical vehicles. New approaches to enhance solubility are emphasized since few suitable methods are available to the formulator of parenteral products.

Most of the product development effort is conducted under contract with the Section staff serving as project monitors. In addition, an intramural formulation laboratory supervised and maintained by this Section is investigating methods to solve drug formulation problems.

This Section supervises a total of four contracts: two analytical contracts; one formulation research contract; and one small-scale production contract. An additional contractor for the development of radioimmunoassays of otherwise poorly characterized drugs will be added to the Section's responsibilities during the next fiscal year.

3. Clinical Products Section - Head, Mr. Larry M. Kleinman

The Clinical Products Section was established in FY 1978. This Section is responsible for production, procurement and distribution of pharmaceutical dosage forms for clinical trial. The activities of the Section involve multi-contract responsibilities and management of several million dollars in drug acquisition expenses. The Section interacts with the Drug Regulatory Affairs Section of the Investigational Drug Branch, CTEP, DCT, NCI.

The major functions include management of three pharmaceutical production contracts; management of a clinical drug storage, distribution and computerized inventory contract and the direct purchase of all clinical products used in the various DCT Programs.

Investigational product literature in the form of pharmaceutical data sheets is prepared by the Section. Product label specifications and designs are also prepared by this Section.

In addition to the management of the pharmaceutical and distribution resource contracts, this Section manages a sizeable intramural budget for the direct purchase of chemicals and formulated products. During this reporting period, the drug purchase expenditures were in excess of $5,000,000.00. A significant amount of staff time is expended in this area in preparing purchase specifications, award justifications and periodic budget projections throughout the year. Several types of NIH contract mechanisms are utilized for these procurement actions including bids from suppliers, blanket purchase orders, direct purchase contracts, etc.

Goals and Accomplishments

The Branch successfully accomplished its objectives during this reporting period by providing efficient chemical and pharmaceutical services to the programs of the Division of Cancer Treatment. For example, more than 82 kg of high quality methotrexate was produced for high dose studies. This large quantity of drug could not be obtained commercially in sufficient quantity to prevent delays in the ongoing Clinical Programs. The contract preparation of this chemical and the ultimate formulation by other Branch contractors resulted in a considerable financial savings to the Division. Other chemicals of high quality produced by the chemical prep laboratories included PALA, 26 kg; methyl CCNU, 48 kg; AMSA (NSC-249992); pentamethylmelamine (NSC-118742); etc.

The Analytical and Product Development Section continued to provide superior analytical contract services to the program. In addition to the evaluations of chemical and formulated products, the analytical contractors assisted the prep lab contractors with analytical problems. Two such problems which were resolved through this collaboration with contractors involved the drugs PALA and 5-methyltetrahydrohomofolic acid.

The Analytical and Product Development Section completed development on several new investigational products including a difficult formulation problem with the unstable compound 6-selenoguanosine. Other products developed included PALA, pentamethylmelamine, DON (NSC-7365) and five other products.

Another achievement of note of the Analytical and Product Development Section involved the development of an ultrafiltration technique for the removal of pyrogens in parenteral products. Pyrogen contamination has become a considerable problem with the advent of high dose drug administration. The ultrafiltration technique has allowed recovery of expensive drug substances such as calcium leucovorin and methotrexate that would have otherwise been destroyed.

The pharmaceutical production activity, under the management and supervision of the newly formed Clinical Products Section, has successfully maintained a clinical inventory of high quality investigational products for the Division of Cancer Treatment Program. The contractors in this Section produced almost one million parenteral units and more than 1.6 million oral dosage units (tablets and capsules). The investigational product cis-diamminedichloroplatinum (II) has the highest clinical interest having 312,000 vials produced during this reporting period. Also produced in large quantity were 90,000 vials of methotrexate; 87,000 vials of daunorubicin; 42,288 vials of calcium leucovorin; and 50,631 vials of 5-azacytidine.

The computerized clinical drug inventory system, under the management and supervision of the Clinical Products Section, is now fully operational. The system provides updated computerized inventory on all clinical products and historical procurement and distribution data as required on these products. The system has answered numerous queries on drug cost, foreign shipment information, drug distribution trends, etc.

The radiolabel synthesis contractors were considerably reduced in operational budget, but still managed to procure 29 radiolabeled materials and make over 157 shipments during this reporting period.

A preparation laboratory contractors meeting was held during this reporting period to discuss new techniques and procedures. The meeting was highly successful and beneficial to the contractors and ultimately to the program.

The Branch continues to meet all new challenges with interest and enthusiasm. Our objective is to be prepared to meet all challenges and to maintain a contract capacity to provide efficient and productive pharmaceutical support to the Division of Cancer Treatment Program. For instance, new areas of endeavor are being undertaken that require considerable effort within the Pharmaceutical Resources Branch. The handling and formulation of Schedule I drugs such as heroin and Δ^9-THC is being pursued. The identification and evaluation of Mexican laetrile products was accomplished and published during this reporting period.

Finally, the U.S.P.H.S. Commendation Medal was awarded to Mr. James C. Cradock, Senior Pharmacist and Head, Analytical and Product Development Section, Pharmaceutical Resources Branch.

The Branch actively participated in several professional meetings during this reporting period:

1. Academy of Pharmaceutical Sciences, American Pharmaceutical Association

    a. Rahman, A. and Cradock, J.C. Carrier Mediated Drug Delivery: Physiologic Disposition of Adriamycin-DNA Complex in Rodents, 22nd National Meeting, Phoenix, Arizona, November 17, 1977.

    b. Poochikian, G.K. and Cradock, J.C. Interaction of Hydroxybenzoates with the Antitumor Agent Chartruesin, 125th APhA Annual Meeting, Montreal, Canada, May 17, 1978.

2. American Association for the Advancement of Science, Section on Pharmaceutical Sciences

    a. Davignon, J.P., Venditti, J.M. and Schein, P. Laetrile - An Assessment (Symposium), Washington, D.C., February 15, 1978.

Publications by Staff

1. Cradock, J.C., Guder, L.A., Francis, D.L. and Morgan, S.L.: Reduction of Pyrogens--Application of Molecular Filtration. J. Pharm. Pharmac. 30:198-199, 1978.

2. Cradock, J.C., Kleinman, L.M. and Davignon, J.P.: Intrathecal Injections--A Review of Pharmaceutical Factors. Bull. Parenteral Drug Assoc. 31:237-247, 1977.

3. Cradock, J.C., Kleinman, L.M. and Rahman, A.: Evaluation of Some Pharmaceutical Aspects of Intrathecal Methotrexate Sodium, Cytarabine and Hydrocortisone Sodium Succinate. Am J. Hosp. Pharm. 35:402-406, 1978.

4. Cheng, C.C., Engle, R.R., Hodgson, J.R., Ing, R.B., Wood, H.B., Yan, S-J and Zee-Cheng, Robert K.Y.: Absence of Mutagenicity of Coralyne and Related Antileukemic Agents. A Structural Comparison with the Potent Carcinogen 7,12 dimethylbenz[a]anthracene. *J. Pharm. Sci.* 66, No. 12, 1781-1783, 1977.

5. Davignon, J.P.: Contaminated Laetrile: A Health Hazard. (Letter) *New Eng. J. Med.* *297(24)*:1355-1356, December, 1977.

6. Davignon, J.P., Trissel, L.A., Kleinman, L.M. and Cradock, J.C.: Pharmaceutical Assessment of Amygdalin (Laetrile) Products. *Cancer Treat. Rep.* *62*:1, January, 1978.

7. Driscoll, J.S., Melnick, N.R., Quinn, F.R., Lomax, N., Davignon, J.P., Ing, R.B., Abbott, B.J., Congleton, G. and Dudeck, L.: Psychotropic Drugs as Potential Antitumor Agents: A Selective Screening Study. *Cancer Treat. Rep.* *62*:1, January, 1978.

8. Flora, K.P., Cradock, J.C. and Ames, M.M.: A Simple Method for the Estimation of Amygdalin in Urine. *Res. Com. Chem. Path. and Pharmacol.* In Press.

9. Grant, J.P., Cox, C., Kleinman, L.M., et al: Serum Hepatic Enzyme and Bilirubin Elevations During Parenteral Nutrition. *Surg. Gynecol. Obstet.* *145*:573-580, 1977.

10. Rahman, A., Cradock, J.C. and Davignon, J.P.: Dissolution and Absorption of the Antineoplastic Agent Ellipticine. *J. Pharm. Sci.* *67*:611-614, May, 1978.

11. Trissel, L.A., Kleinman, L.M., Davignon, J.P. and Cradock, J.C.: Investigational Drug Information - Daunorubicin Hydrochloride, Streptozocin. *Drug Intell. Clin. Pharm.* In Press.

Publications by Contractors

1. Bannister, S.J., Sternson, L.A., Repta, A.J. and James, G.W.: Measurement of Free-Circulating Dis-Dichlorodiammineplatinum (II) in Plasma. *Clin. Chem.* *23*:2258, 1977.

2. Bourne, D.W.A., Higuchi, T. and Repta, A.J.: Acetylacronium Salts as a Soluble Pro-Drug of the Cytotoxic Agent Acronine. *J. Pharm. Sci.* *66*: 628-633, 1977.

3. Chen, C.R., Fong, M.T., Fujiwara, A.N., Henry, D.W., Leaffer, M.A., Lee, W.W. and Smith, T.H.: Synthesis of Daunorubicin-14-^{14}C and Adriamycin-14-^{14}C. *J. of Labelled Compounds and Radiopharmaceuticals.* *14(1)*:111-117, 1978.

4. Hansen, A.B., Kreilgard, B., Huang, C.H. and Repta, A.J.: Analysis of the Prodrug 7-Acetylacroninium Perchlorate in Presence of the Parent Compound Acronine. J. Pharm. Sci. 67:237, 1978.

5. Jee, J., Yoshikawa, F. Pont, L., Cheung, A. and Lim, P.: Assay of Amygdalin Dosage Forms from Mexico. J. Pharm. Sci. 67(3), March, 1978.

6. Repta, A.J., Dimmock, J.R., Kreilgard, B. and Kaminski, J.J.: Hydrolysis Mechanism of 7-Acetylacroninium Perchlorate, a Novel Prodrug of Acronine. J. Pharm. Sci. 66:1501, 1977.

Patents by Contractors

1. Ellard, J.A., U.S. Patent 4,080,325, Synthesis of Methotrexate, Issued March 21, 1978.

CHEMICAL RESOURCES SECTION

PHARMACEUTICAL RESOURCES BRANCH

Scope

The function of the Chemical Resources Section is to procure bulk chemicals and drugs for confirmational and other testing, toxicological and pharmacological evaluation, and clinical trials in humans; and to procure radiolabeled materials for pharmacology, clinical pharmacology and related studies. The function of the Section is achieved through the management and supervision of a contract program for the preparation of various quantities of bulk chemicals and drugs and radiolabeled materials, and the acquisition of commercially available chemical substances.

Staff

The staff of the Chemical Resources Section consists of one professional person and a secretary. The professional staff member serves as the project officer on the various contract activities of the Section.

Preparative Activities

The resynthesis or preparation laboratories are, in the strictest sense, service laboratories, and are designed and selected to prepare known chemicals and bulk drugs which are needed by the Program. The compounds selected for preparation are not readily available in the quality or quantities needed from the original supplier or on the open market.

These laboratories are also used to obtain data for the preparation of the necessary quantities of clinically-important chemicals and to develop the most economical means for their preparation. It should be pointed out that many methods of synthesis which are practical for small quantity are not technically feasible or economically practical when used for a large scale synthetic operation or for radiolabel synthesis. The conversion of small-scale to large-scale production often requires developmental studies which are conveniently carried out by the preparation laboratories. Solubility and stability studies, and cost data are also provided by these contractors. These contractors have, on occasion, provided materials in formulated dose form.

The increased emphasis in clinical investigations has caused a change in the preparation activities. The increased effort being devoted to toxicological and clinical studies has resulted in the need for larger quantities of the compounds prepared by this program. Also, the decision to evaluate a larger number of compounds demonstrating confirmed activity in the initial screening in the tumor panel has also resulted in the need for larger quantities of the compounds being resynthesized for preclinical evaluation. This change in emphasis has been handled by the preparation laboratories without difficulty.

The preparation laboratories, taken collectively, provide the means of obtaining nearly any type of chemical compound, regardless of structure, and the ability of providing large quantities of very high purity drugs.

1. Bulk Chemicals and Drugs

The compounds chosen for resynthesis at the preparation laboratories are assigned for resynthesis for five major reasons: (a) clinical investigations; (b) preclinical toxicological and pharmacological evaluation; (c) maintaining a stock of chemicals of clinical and preclinical interest; (d) completion of screening or confirmational testing; and (e) use as intermediates in further synthesis. The compounds so chosen are placed on priority lists based upon their relative importance, with clinical compounds given top rating. The quantity of a given material to be resynthesized may vary from 1 gram to 200 kilograms. Factors governing the amounts depend upon: use, ease of preparation, stability, and cost. Materials of high quality are prepared in the preparation laboratories. Every effort is made to safeguard the patient in the quality of the drugs used.

The Chemical Resources Section, through the preparative laboratories, directed the resynthesis of 204 compounds totaling 535 kilograms during this report period. Included in this group were 21 compounds scheduled for or undergoing clinical evaluation totaling 205 kilograms. In addition to the compounds resynthesized, 62 new compounds were also prepared by the preparative laboratories. The Section also purchased 5 compounds scheduled for or undergoing clinical evaluation totaling 144 kilograms. In addition, the Section obtained as gifts from various organizations a total of 3 compounds totaling 18.7 kilograms which were scheduled for toxicological or clinical evaluation.

During this report period, large quantities of Methotrexate (NSC-740) (82 Kg), Methyl-CCNU (NSC-95441) (48 Kg) and PALA (NSC-224131) (26 Kg) were prepared by the preparation laboratories. The Methotrexate was prepared via the new procedure devised by Southern Research Institute and developed by the preparation laboratories. The Methotrexate prepared has been of very high quality and it has been prepared at a very reasonable cost (a substantial savings over the previous purchase price). The procedure for the preparation of high quality PALA in large quantities has been developed by the preparation laboratories. In addition, the preparation laboratories prepared the large quantity of the two key intermediates required for the preparation of the large quantities of PALA needed for preclinical and clinical studies. These key intermediates were not available in the quality or quantity needed from other sources. In addition, the preparation laboratories responded rapidly to the preparation of large amounts of several other high priority materials needed by the Program, such as, AMSA (NSC-249992), Dichloromethotrexate (NSC-29630), Pentamethylmelamine (NSC-118742), Indicine-N-oxide (NSC-132319), and AZQ (NSC-182986).

The Section directed nine contract laboratories for the preparation of materials needed by the Program (Table II).

TABLE II

CONTRACT LABORATORIES FOR PREPARATION
OF CLINICAL AND EXPERIMENTAL COMPOUNDS

Contractor	Investigator	Contract No.
Aldrich Chemical Company	Szabo	NO1-CM-87202
Ash Stevens, Inc.	Ash	NO1-CM-57012
Ash Stevens, Inc.	Markovac	NO1-CM-87204
Cordova Chemical Company	Olsen	NO1-CM-87201
Monsanto Research Corporation	Ellard	NO1-CM-67046TQ
Pharm-Eco Laboratories, Inc.	Draper	NO1-CM-87200
Starks Associates, Inc.	Starks	NO1-CM-87203
Starks Associates, Inc.	Schultz	NO1-CM-53769
Warner-Lambert/Parke-Davis	McMillan	NO1-CM-87124

2. Radiolabeled Materials

The Section coordinates the procurement and distribution of radiolabeled chemicals and drugs for pharmacological and drug distribution studies. Materials not available from commercial sources are prepared by contract preparative laboratories. These contracts also handle the storage and distribution and perform the necessary analytical work for all labeled materials, whether prepared under the contracts or acquired from other sources. All requests for radio-active materials are reviewed by a committee in relation to proposed use prior to assignment to the contracts for procurement. All materials are checked for purity prior to shipment and repurified if necessary before shipping. A total of 29 radiolabeled materials (Table III) have been procured from all sources. One hundred and fifty-seven shipments of radio-active substances were made during this report period.

TABLE III

RADIOLABELED MATERIALS PROCURED

NSC No.	Compound	Label
740	Methotrexate	^{3}H (3',5')
740	Methotrexate	^{14}C (N-methyl)
752	6-Thioguanine	^{14}C (8)
755	6-Mercaptopurine	^{14}C (8)
3590	Leucovorin	^{3}H (General)
8806	Melphalan	^{14}C (Ring)
8806	Melphalan	^{14}C (Ethylene)
8806	Melphalan	^{3}H (General)
13875	Hexamethylmelamine	^{14}C (2,4,6)
26271	Cytoxan	^{14}C (Ethylene)
51143	2,3-Dihydro-1H-Imidazo(1,2b) pyrazole (IMPY)	^{14}C (2,3)
67574	Vincristine	^{3}H (General)
71795	Ellipticine	^{14}C (1)
79037	1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)	^{14}C (cyclohexyl)
79037	1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)	^{14}C (Ethylene)
82151	Daunorubicin	^{14}C (Uniform)
95441	1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea (Methyl-CCNU)	^{14}C (Ethylene)

TABLE III (Cont'd)

RADIOLABELED MATERIALS PROCURED

NSC No.	Compound	Label
95466	1-(2-Chloroethyl)-2-(2,6-dioxo-3-piperidinyl)-1-nitrosourea (PCNU)	^{14}C (Ethylene)
95466	1-(2-Chloroethyl)-2-(2,6-dioxo-3-piperidinyl-1-nitrosourea (PCNU)	d (Ethylene)
118742	Pentamethylmelamine	^{14}C (2,4,6)
118742	Pentamethylmelamine	^{14}C (N-methyl)
123127	Adriamycin	^{14}C (14)
153878	Maytansine	^{3}H (General)
178248	Chlorozotocin	^{14}C (Glucose)
178248	Chlorozotocin	^{14}C (Ethylene)
224131	PALA	^{14}C (Carbonyl of Acetyl)
239387	Maytanacine	^{14}C (Acetyl)
261037	α-(Methoxymethyl)-2-nitro-1H-imidazole-1-ethanol	^{14}C (2)
263164	erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA)	^{14}C (8)

The Section directed two contracts devoted to the procurement of radiolabeled materials (Table IV).

TABLE IV

CONTRACT LABORATORIES FOR PREPARATION OF RADIOLABELED MATERIALS

Contractor	Investigator	Contract No.
Research Triangle Institute	Kepler	NO1-CM-67121
SRI International	Leaffer	NO1-CM-63776

Contractors Workshop

During this report period a Preparation Laboratory Contractors Workshop was held with representatives from all the Preparation Laboratory Contractors, the Radiolabeled Contractors and the Analytical Contractors, in attendance. The major objective of this meeting was for the different contractors in these areas of endeavor to discuss mutual problems, techniques and procedures. In addition, the Section Staff had an opportunity to review administrative procedures and to inform the contractors of changes in the Program. Invited speakers made presentations of interest to the group and the contractors discussed topics of mutual interest. A favorable response to this workshop was expressed by all in attendance and the contractors requested that additional workshops of this type be held. Additional workshops of this type are being planned.

ANALYTICAL AND PRODUCT DEVELOPMENT SECTION

PHARMACEUTICAL RESOURCES BRANCH

The analytical chemistry and dosage form development activities are merged into one Section within the Pharmaceutical Resources Branch. Major responsibilities of the Section fall into two distinct categories:

I. Analytical Chemistry

a. Development of suitable methodology for the analysis and characterization bulk chemicals, and pharmaceutical dosage forms.

b. Assess the quality of bulk chemicals and pharmaceutical dosage forms used in the Developmental Therapeutics Program.

c. Development of specifications for use in procurement of bulk chemical agents.

d. Serve as a repository for all methods of preparation and analytical characterization of bulk chemicals. The data are summarized in a Chemical Information Sheet and made available to investigators using this material.

II. Dosage Form Development

a. Characterization of the pharmaceutical properties of new chemical agents, development of dosage forms, and evaluation of chemical and physical changes under simulated use conditions.

b. Development of new approaches for enhancing solubility and stability of selected antitumor agents.

This Section is staffed by one pharmacist, one analytical chemist, one staff fellow, one visiting fellow, and one secretary.

A. Analytical Chemistry

Clinical and preclinical evaluations of antitumor agents require availability of bulk chemicals and pharmaceutical dosage forms of highest quality. These materials must be subjected to rigorous assessment of their identity, purity and quantitative composition by definitive analytical methods. Analyses are conducted by contract laboratories independent of chemical or formulated drug suppliers. The analytical data are utilized for a number of chemical and pharmaceutical applications within the Pharmaceutical Resources Branch including:

1) Preformulation assessment of solubility and stability as well as analytical characterizations that are of benefit in the design of dosage form development studies.

2) Establishment of procurement specifications for bulk chemicals acquired on a bid basis.

3) Assembly of analytical data on bulk drug substances and pharmaceutical dosage forms for submission to the Food and Drug Administration. Analytical data are retained on all bulk materials used in clinical trial under the sponsorship of the Division of Cancer Treatment.

In addition, information acquired from these analytical laboratories is condensed into a "Chemical Information Sheet." This compilation is a part of the Clinical Brochure which is distributed to the various investigators. The data provide information on the characterization of the drug substance, serve as a guide for safe handling during storage and shipping, and supply preliminary chemical and solubility data potentially applicable to extraction and estimation of the drug in biologic samples. In addition, partition coefficients are determined in octanol-water systems for application to structure activity studies.

The determination of the purity of a new chemical entity is often an analytical research problem requiring the use of a combination of sophisticated techniques as well as consideration of the stability of the compound and its possible contaminants or decomposition products. Among the techniques that are most commonly used in drug analysis are: spectroscopic (ultraviolet, visible, infrared and nuclear magnetic resonance), chromatographic (paper, thin layer, high pressure liquid, and gas-liquid) and titrimetric (aqueous and potentiometric). In addition to the above, elemental microanalysis is carried out. Where applicable, optical rotation, optical rotatory dispersion, refractive index, functional group assay, degree of hydration or solvolysis determinations and the preparation of derivatives are performed. Chromatographic procedures are most useful in the detection of impurities. In certain instances, the impurities are isolated and characterized by comparison of chromatographic mobility with known substances and spectroscopic assessments similar to those used for the bulk drug.

A reference standards program has been in operation for two years. One bulk lot is designated as the reference and made available primarily to contractors within the Pharmaceutical Resources Branch. This batch is of high quality and is selected on the basis of chemical purity from two or three lots of comparable size. The analyst compares subsequent batches of bulk drug with the reference using definitive methods.

Assistance was also extended to the formulation facilities and the preparation laboratories with their analytical and formulation problems concerning compounds of interest to the program.

During this year, there were 194 analytical reports on 67 different compounds. These reports consisted of the analysis of 138 lots of bulk chemicals of clinical interest (351.5 kg), six lots of other bulk materials, and 85 lots of clinical formulations.

Although a large number of chemically diverse compounds were analyzed this past year, the studies on amygdalin and PALA carried out at Stanford Research Institute deserve special mention. Samples of amygdalin injection and tablets were obtained from U.S. Customs and evaluated for pharmaceutical acceptability in the event a clinical trial might be initiated. Eight lots of pharmaceutical dosage forms were each evaluated by several instrumental methods including infrared, ultraviolet and proton magnetic resonance spectroscopy. Spectral data indicated that the tablets contained D-amygdalin and the injectables, a mixture of D and L-amygdalin approximating a 1:1 ratio. An HPLC method was developed and used to quantitate amygdalin. By this method all batches were found to be markedly subpotent and outside the minimum range commonly used for acceptability. In addition, the ampoules contained an average amygdalin content of 45-55% plus substantial quantities of amygdalinamide and amygdalin acid. The identity of these impurities was verified by comparison with authentic samples synthesized by the contractor. All eight lots were considered to be of poor pharmaceutical quality and unacceptable for clinical use.

PALA is an amino acid analogue that was difficult to analyze with non-degradative techniques since it does not contain a chromaphore. Three methods have been developed to quantitate PALA and have been applied to monitor the quality of the bulk material and pharmaceutical dosage form. In addition to a rather non-specific titrimetric method, proton and P^{31} NMR plus an HPLC method have been developed. The latter procedure uses end absorption at 208 nm to quantitate the separated PALA.

The Section directed two analytical-quality control contract laboratories (Table V). In addition to the two analytical laboratories, elemental analyses are obtained from Micro-Analysis, Inc., under a purchase agreement.

TABLE V

CONTRACT LABORATORIES FOR ANALYTICAL-QUALITY CONTROL

Contractor	Investigator	Contract No.
Midwest Research Institute	Haggerty	NO1-CM-33722
Stanford Research Institute	Lim	NO1-CM-33723

B. Dosage Form Development

Formulation development usually commences when an agent passes Decision Network IIA. The first approach to development involves an evaluation of existing physical and chemical data as well as in vivo route and regimen studies in rodents. These results serve to define the scope of the formulation problem. Relatively straight-forward development projects are assigned directly to the contract laboratory equipped with production capabilities. The contractor is provided with such information as chemical data, estimated dose and intended route of administration. Consultation with the Project Officer is carried out regarding formulation approaches. The chemical is subjected to solubility and stability studies in a selected group of physiologically acceptable vehicles. An analytical method is usually devised for detection of the parent compound in the presence of excipients and/or degradation products. The influence of pH, temperature, light, oxygen, packaging components and pharmaceutical additives is assessed on the stability of drug solutions. Pilot batches are then prepared under conditions simulating production scale. Physical and chemical stability of the dosage form is assessed under accelerated use conditions.

Some of the drugs recommended for development as intravenous dosage forms are poorly water soluble. Since biologically suitable solubilization techniques are limited, low solubility has been a problem in preparation of parenteral dosage forms. Newer approaches have been investigated under a contract with the University of Kansas and in the Pharmaceutical Resources Branch formulation laboratory. NSC-137679, 6-selenoguanosine, has been formulated as a lyophilized dosage form containing ascorbic acid to retard hydrolysis. Since the diselenide oxidation product is much less soluble than the parent compound, addition of the antioxidant reduced degradation and circumvented a solubility problem. Another significant accomplishment by this contractor is the development of a sensitive HPLC procedure for cis-diamminedichloroplatinum and the application of this method to assess the stability of this agent in a variety of infusion solutions. Provided that the infusion fluid contains at least 0.1% sodium chloride, cis-diamminedichloroplatinum is stable for 24 hours. Research efforts in the formulation laboratory are described in project Z01-CM-03584-05.

Unique capabilities in dosage form development and production have enabled the University of Iowa to play an increasingly important role in our program. The contractor provides sophisticated product development expertise in areas of intravenous and oral dosage forms plus the production capacity to meet requirements for large animal toxicology or phase I clinical trial. During the past year they have completed development studies and produced dosage forms of several drugs designated as high priority by the Division of Cancer Treatment including NSC-224131, PALA; NSC-118742, Pentamethylmelamine HCl and NSC-7365, 6-diazo-5-oxo-L-norleucine. Also, five other development projects and twenty additional production assignments were completed.

The Section was also heavily involved in the detection, surveillance and elimination of pyrogens from certain dosage forms and bulk chemicals. Most of the compounds were folate analogues. The level of pyrogens was low; however, the recent use of very high doses (3-15 g) of methotrexate, calcium leucovorin, dichloromethotrexate, etc., resulted in administration of therapeutically significant levels of pyrogens. An ultrafiltration technique was developed in conjunction with Ben Venue Laboratories that resulted in at least two orders of magnitude reduction in pyrogens without markedly affecting drug concentrations. This method is rapid (at least 10 liters/hour output), and the ultrafiltrate may be successively sterilized by membrane filtration and then aseptically subdivided into vials.

All experimental formulations are submitted for evaluation and confirmation of antitumor activity. Simultaneously, with these studies, the dosage forms are sent to an independent laboratory for chemical and/or biological analyses. The toxicological results and the accumulated pharmaceutical development data serve as a basis for determining the starting human dose and the ultimate dosage form to be used in the initial clinical studies. Several dosage strengths of the same investigational drug may be prepared provided the drug concentration remains within the solubility limits of the active ingredient.

During 1976, 28 projects for dosage form development were assigned. Fourteen of these involved new drug research and twelve assignments were for modification of existing formulations.

TABLE VI

CONTRACTS FOR DOSAGE FORM DEVELOPMENT

Contractor	Investigator	Contractor No.
Iowa, University of	Wurster	NO1-CM-43743
Kansas, University of	Higuchi	NO1-CM-23217

CLINICAL PRODUCTS SECTION

PHARMACEUTICAL RESOURCES BRANCH

Scope

The responsibilities of the Clinical Products Section include the following:

1. Production, packaging and labeling of investigational dosage forms;
2. Procurement by direct purchase of formulations for clinical use;
3. Shelf life surveillance of all clinical dosage forms;
4. Preparation of FDA-IND attachments 1, 2, 3, 4, 5 and 7 for subsequent submission to the Food and Drug Administration;
5. Preparation of Investigational Drug - Pharmaceutical Data sheets for Clinical Brochures, IND's, etc;
6. Management of a computerized clinical drug inventory system;
7. Storage and distribution of clinical dosage forms.

Staff

The staff of the Clinical Products Section consists of two senior professionals and one purchasing clerk. The professional staff members are PHS Commissioned Corps Pharmacists and serve as project officers on the various contract activities of the Section.

Production and Packaging of Investigational Drugs

The Section has the responsibility to provide adequate quantities of formulated drugs to the clinical programs. The production of investigational dosage forms is mainly performed by contract agreements with qualified pharmaceutical firms. The production output of investigational dosage forms for the period covered by this report remained at a high level of effort. The formulation contractors produced a combined total of 864,579 parenteral doses and 39,034 bottles of tablets and capsules (1,616,520 oral doses). Production output which is dictated by clinical needs remained constant during this reporting period. The production contractors have been able to meet the program's needs for a variety of formulated products. Four contracts were monitored by the Clinical Products Section.

TABLE VII

CONTRACTS MONITORED BY THE CLINICAL PRODUCTS SECTION

Contractor	Investigator	Contract No.
Ben Venue Laboratories, Inc.	Wickes	N01-CM-67109
Philips Roxane Laboratories, Inc.	LaRowe	N01-CM-67053
Elkins-Sinn, Inc.	Reese	N01-CM-67103
Flow Laboratories, Inc.*	Dembeck	N01-CM-67088

*Responsible for clinical drug storage and distribution and operation of a computerized clinical drug inventory system.

Several investigational products are provided by pharmaceutical companies on a cost sharing basis. These products are usually provided in return for access to clinical data generated by the studies. The dosage forms are prepared from specifications which are established by mutual agreement between the Branch and the manufacturers. During the period of this report, the following companies supplied a total of 124,370 parenteral units as well as 2,629 bottles of solid oral dosage forms as part of this joint effort:

Bristol Laboratories, USA	NSC-157365	Neocarzinostatin
Eli Lilly and Company, USA	NSC-143095	Pyrazofurin
Sandoz, USA	NSC-122819	VM-26
Sandoz, USA	NSC-141540	VP16-213
Wellcome Foundation, England	NSC-220537	Corynebacterium Parvum
Janssen Pharmaceuticals, USA	NSC-177023	Levamisole
Richardson-Merrell, USA	NSC-151466	Enclomiphene
Rhodia, Inc., France	NSC-164011	Rubidazone

Purchase of Dosage Forms

Another function of the Section involves the direct purchase of formulated products. These items fall into two categories: (1) investigational products; and (2) commercial products. Investigational products are usually manufactured according to NCI specifications and sold to NCI on a cost reimbursement basis. Commercial products are items available for open purchase. Procurement of all direct purchased dosage forms is handled through the NIH Procurement Branch. Several procurement mechanisms are used depending on such factors as the type of product, the quantity required and the priority for clinical need.

A total of 538,718 injectable units and 20,432 bottles of tablets and/or capsules (1,888,200 oral doses) of direct purchased items at a cost of $4,249,077.00 were obtained during this reporting period. All suppliers are required to provide a certificate of analysis on each lot of drug purchased. The data are reviewed prior to release for clinical use.

The table below summarizes the procurement activities of the Clinical Products Section.

TABLE VIII

DRUG ITEMS PROCURED

	Capsules/Tablets	Injectables
Produced by Contractors	1,616,520	864,579
Contributed or Cost Sharing	159,150	154,415
Purchased Investigational Units	139,000	125,187
Purchased Commercial Units	2,922,125	490,066
	4,836,795	1,634,247

The Clinical Products Section is responsible for the disbursement of funds from CAN #8-8322759. This CAN is used for acquisition of products intended for clinical trial which include natural products, bulk chemicals and clinical products. Emergency drug requisition not previously budgeted for were purchased from the CAN. Also, several of the high priority items were also procured, for example, PALA (and intermediates), Methotrexate, Calcium Leucovorin and cis diamminedichloroplatinum.

Careful monitoring of procurement is essential since the operational budget of $5,128,000 (FY 78) is below the projected requirements.

Quality Assessment of Clinical Dosage Forms

Each lot of formulated drug must pass quality assessment criteria prior to release for clinical trials. The Clinical Products Section in collaboration with the other two Sections in the Branch, maintains a strict assessment program designed to insure that each batch of formulated material meets the criteria for strength, quality and purity. Parenteral products are tested for potency, sterility, safety, pyrogenicity, etc., while oral formulations are tested for potency, content uniformity, weight variation, hardness, disintegration, dissolution, etc. All products are tested for potency by the formulator with random testing being conducted by an analytical contractor. All new dosage forms and all first production batches of a new formulator are routinely submitted to an analytical contractor to verify the accuracy of the formulator.

The pharmaceutical contractors are requested to perform compatibility and stability studies on reconstituted and diluted solutions of parenteral investigational dosage forms. These studies are designed to simulate the conditions found in the clinical setting. The data generated is assembled and is summarized in the format of an Investigational Drug - Pharmaceutical Data sheet which is incorporated into the Clinical Brochure.

The Clinical Products Section maintains a shelf life surveillance program on all lots of contractor produced investigational dosage forms. Random samples of each lot are stored under specified controlled conditions and are assayed for chemical and/or physical change. Three stability schedules are used for shelf life surveillance; (1) Schedule A, designed to provide stability data under accelerated conditions; (2) Schedule B, designed for studies of repeated formulations; and (3) Schedule C, assigned to products that have been prepared more than three times. The last schedule involves the retention of samples for assays to be performed on request. All schedules provide a minimum of four years surveillance.

Preparation of Investigational Drug - Pharmaceutical Data Sheets

The Clinical Products Section is responsible for the preparation of pharmaceutical data sheets for all investigational dosage forms used in the program. The data sheets are used in the preparation of Clinical Brochures and are disseminated to health-care practitioners. These data sheets provide product descriptions, reconstitution and stability information and precautions in handling the products.

FDA-IND Submissions

The Clinical Products Section also prepares Investigational New Drug (IND) Attachments I, II, III, V and VII (labels) for submission to the Investigational Drug Branch, CTEP, DCT, NCI for FDA-IND filing. These attachments contain manufacturing, control, packaging and labeling data required for the investigational dosage forms. During the reporting period, the Section prepared attachments for seven original IND's and numerous amended IND's.

Storage and Distribution of Clinical Dosage Forms

During the reporting period, a total of 1,572,991 drug items (vials and bottles) were received and inventoried for subsequent worldwide distribution. During the same period, 1,584,808 drug items were distributed averaging 47 individual shipments daily.

During this report period, an average of 117 different formulations were maintained on the clinical drug inventory. These items included investigational and commercial products.

Significant Accomplishments

The pharmaceutical production contractors of the Section were able to meet an increased clinical demand for several high interest drugs.

The production contractors produced over 312,000 vials of cis-diamminedichloroplatinum (II); over 90,000 vials of methotrexate (all strengths); 87,000 vials of daunorubicin; 42,288 vials of calcium leucovorin; and 50,631 vials of 5-azacytidine.

The storage and distribution contractor (Flow Laboratories) acquired the computerized inventory management system from CTEP. This transition between Programs went well and in two months the Contractor was up to full speed regarding turnover time for special queries.

The Clinical Products Section has been developing a dialog with Pharmacists, Physicians and other Health Care Practioners participating in Clinical Oncology Programs at the various cancer centers. This dialog has resulted in a timely exchange of information beneficial to both parties. This information is especially important with drugs undergoing phase I and phase II clinical trial.

This activity involved the distribution of over 200 packets of Pharmaceutical Data Sheets and the handling of 10-15 inquiries per week.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03584-06 PRP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Research in the Dosage Form Development of New Antitumor Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. C. Cradock	Head, Anal. & Product Dev. Sect.	PRB NCI
OTHER:	J. P. Davignon	Chief, Phar. Res. Br.	PRB NCI
	K. P. Flora	Staff Fellow	PRB NCI
	G. Poochikian	Visiting Fellow	PRB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Pharmaceutical Resources Branch

SECTION
Analytical and Product Development Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project describes the intramural formulation activities with potential antineoplastic agents. Several of the agents investigated were poorly water soluble symmetrical compounds that contained at least two identical labile groups NSC-182986, aziridinyl benzoquinone; NSC-172112, spirohydantoin mustard; and diacetylmorphine hydrochloride. High performance liquid chromatographic methods were developed for NSC-182986 and diacetylmorphine using reverse phase columns. Hydrolysis was studied over a wide pH range and degradation products were characterized.

Additional studies involved a sensitive method for the estimation of amygdalin in human urine. A degradative method was developed that involved hydrolysis by β glucosidase plus alkali to yield glucose, cyanide and benzaldehyde. The latter was extracted into methylene chloride and measured by ultraviolet absorbance.

General Objectives:

1. Study new formulation approaches with potential utility for those compounds posing difficult drug delivery problems.
2. Evaluate dosage form problems presented by clinical and laboratory investigators.
3. Develop suitable analytical methods to monitor the stability of drugs and pharmaceutical dosage forms.

Specific Objectives:

1. Assessment of the stability of aziridinyl benzoquinone (AZQ, NSC-182986) in aqueous solution and development of a suitable parenteral dosage form.
2. Development of a simple sensitive method for detection of amygdalin in human urine.
3. Development of a parenteral dosage form of spirohydantoin mustard.
4. Evaluation of the hydrolysis of diamorphine HCl and development of a suitable parenteral dosage form.
5. Evaluation of pharmaceutical additives in the low temperature vacuum drying method.
6. Formulation studies on the efficacy of an adriamycin-DNA complex.

Major Findings:

1. Aziridinyl Benzoquinone, NSC-182986 (Dr. Poochikian)

   This quinone containing alkylating agent exhibited activity vs a wide spectrum of experimental tumor models including solid CNS implants (Khan and Driscoll, J. Med. Chem., 19:313, 1976) and is regarded as a "high priority" agent in the Developmental Therapeutics Program. However, it is poorly water soluble and possesses acid labile aziridine groups. A high performance liquid chromatography method was developed utilizing a reverse phase system to monitor the stability of AZQ vs pH in aqueous solution. Disappearance of AZQ followed pseudo-first order kinetics over the pH range examined (3.0-8.0). Maximum stability was observed at pH 6.0-7.0. At 25±0.1°C, less than 10% decrease in AZQ was observed within 30 hours at pH 6.0 and 7.0. Although the rate of hydrolysis over this pH range was sufficiently slow for administration by slow intravenous infusion, solubility behavior of AZQ presented some difficulties. The modest solubility of AZQ in ethanol ($\sim$ 2 mg/ml) was sufficient to permit preparation of a sterile 10 mg formulation of AZQ powder by the low temperature vacuum drying method. Since AZQ is soluble in dimethylacetamide (> 20 mg/ml), the vial contents are dissolved in 0.5 ml and diluted with 19 volumes of 0.01 M pH 6.5 phosphate buffer to provide a 1 mg/ml AZQ solution that is stable for 24 hours at room temperature.

Hydrolysis products and impurities were separated by preparatory scale HPLC and compared with synthetically prepared compounds. Under acidic conditions the products of hydrolysis were identified principally by NMR and mass spectrophotometry plus chromatographic mobility as 2-aziridinyl-5-(2'-hydroxyethylamino)-3-6-bis-carboethoxyamino)-1,4-benzoquinone and 2,5-di-(2-hydroxyethylamino)-3-6-bis-(carboethoxyamino)-1,4-benzoquinone. A third hydrolysis product was absent in acidic solution, but was a predominant component in alkaline media. The mass spectrum and HPLC elution behavior suggests a polymeric product. Other aziridinyl compounds polymerize in basic solution. Further characterization of this product is in progress. A fourth peak was present in all samples of AZQ, and was characterized as 2,5-diamino-3,6-dichloro-1,4-benzoquinone by comparing its mass spectrum and HPLC behavior with an authentic sample. This compound is an intermediate in the synthesis of AZQ.

The formulation of AZQ has been prepared as outlined above on a production scale, and toxicology studies are in progress.

2. Amygdalin (Dr. Flora)

When clinical trials of amygdalin were under discussion, the need for a simple screening procedure to detect amygdalin in urine was expressed to assure compliance with the protocol. A method was developed to detect D and D,L-amygdalin in human urine at 10 μg/ml or greater.

The procedure involved quantitation of benzaldehyde by UV spectroscopy at 243 nm after enzymic hydrolysis of amygdalin in urine and extraction of the liberated benzaldehyde into methylene chloride. Preliminary studies established experimental conditions including incubation time, appropriate enzyme and drug concentrations, pH, extraction volume, and stereospecificity of the enzyme. The average methylene chloride extraction efficiency from human urine was 89.9±0.3% for various benzaldehyde standards. Plots of concentration vs absorbance for D and D,L-amygdalin added to human urine and treated as described above were linear from 10-75 μg/ml. Hydrolyzed amygdalin rendered a benzaldehyde extraction efficiency of 87.5±0.7% after correction for degree of hydrolysis.

Although D-amygdalin was hydrolyzed somewhat faster than the racemic compound, hydrolysis was complete in both instances in less that one hour at 25°C.

The urinary excretion of amygdalin in mice following oral and intravenous administration of 100 mg/kg was monitored using this method. A total of 69% of the IV dose was recovered in urine as amygdalin equivalents. In contrast, only 19% of the oral dose was detected in urine over 96 hours. In either instance, 96% of the total amount recovered was obtained in the first 24 hours.

3. Spirohydantoin Mustard, NSC-172112 (Dr. Flora)

This agent represents an attempt to improve the treatment of a CNS tumor implant by modifying an anticonvulsant with a bischloroethyl alkylating function. Unfortunately, this poorly water soluble compound also rapidly hydrolyzes in aqueous solution The intent of this study was to assess the magnitude of the instability and to evaluate the various formulation approaches with emphasis on those that would enhance solubility and inhibit hydrolysis. Coulometric-amperometric titration of chloride ion was chosen as a simple, rapid (though non-specific) stability indicating method for following rate of hydrolysis of the alkylating function. Studies by others indicated that liberation of chloride ion parallels loss of alkylating activity and appearance of mono- and di- alcohol derivatives.

Among the approaches evaluated were pH, dilute organic solvents (dimethylacetamide, ethanol, propylene glycol, and polyethylene glycol), complexing agents (albumin, polyvinylpyrrilidone, divalent cations) and surface active agents. The hydrolysis of spirohydantoin mustard in presence of a chemically diverse group of additives was much faster than the minimally accepted rate ($\leq$ 10% decomposition in one hour at 25°C) for an intravenous formulation. Under the same conditions the hydrolysis of mechloremethamine and melphalan was much slower.

Physical separation of reactive components (spirohydantoin mustard and water) until a point just adjacent to the injection port was evaluated. Hydrolysis of spirohydantoin in a 2:1 mixture of neat dimethylacetamide and L-lactic acid was < 0.4% in six hours at room temperature. This mixture was slowly infused into a flowing IV fluid. At the completion of the study, small amounts of solid material (presumably spirohydantoin mustard) were present in the IV line, injection bulb and outflow needle. Due to the observed physical incompatability, this approach was abandoned.

Present studies are attempting to better understand the mechanism of hydrolysis by utilizing more specific methods of analysis. In conjunction with Dr. Kelley of Laboratory of Medicinal Chemistry and Biology, hydrolysis is being studied by gas chromatography of the silylated products. These studies indicate the disappearance of spirohydantoin is pseudo-first order and the half life is about 1/3 of that obtained by chloride ion measurements. Gas chromatography-mass spectral studies indicate that the hydrolysis products are the mono- and di- alcohols.

Additional work is being pursued along two lines: 1) evaluation of the oral route as a means of drug delivery, and 2) preparation of "prodrugs" that might retard hydrolysis, yet release spirohydantoin in plasma.

4. Diamorphine (Dr. Poochikian, Mr. Cradock)

Recent interest in improving pain relief for the terminal cancer patient has resulted in a reawakening of interest in diamorphine hydrochloride. Although the literature contains reports of "stability indicating" methods for this agent and studies of its rate of hydrolysis, the data are inconsistent and techniques are time consuming. A stability indicating high performance liquid chromatographic assay was developed using a reverse-phase (C-18) column and ultraviolet absorbance at 235 nm. This method is quick, sensitive and readily separates diamorphine from its hydrolysis products, 6-acetylmorphine and morphine. Diamorphine is sufficiently stable at 25°C (< 10% decomposition in 24 hours at pH 3.5-5.0 to utilize a multi-dose formulation. However, for long term stability a lyophilized dosage form was developed. This product is intended to be reconstituted, used as needed, and the unused contents discarded after 24 hours. Stability is of special concern in this instance because the pharmacologic response is subjective in nature and the second hydrolysis product (morphine) is also a potent analgesic.

5. Low Temperature Vacuum Drying (Dr. Flora, Mr. Cradock)

Low temperature vacuum drying is an adaptation of lyophilization that facilitates sterile preparation of dry solid parenteral formulations from certain volatile solvents, particularly low molecular weight alcohols. Most drugs formulated by this technique do not contain additives. However, certain additives might be included to optimize pharmaceutical properties by increasing powder mass (urea or polyethylene glycol 4,000), improve solubility by addition of a complexing agent (polyvinylpyrrilidone), or enhancing stability by addition of antioxidants or buffers.

Bruceantin, a novel plant product formulated by low temperature vacuum drying, appears to degrade (~ 10% decomposition at -10°C) within one year when stored in the deep freeze.

An HPLC method was developed to separate bruceantin from its degradation products and the influence of several pharmaceutical additives on stability of the vacuum dried formulation of bruceantin was evaluated. Within three months substantial (~ 35%) decomposition was evident at room temperature in the bruceantin vials. However, inclusion of one or three mole equivalents of ascorbic acid resulted in only 8 and 3% decomposition, respectively. Similar trends are evident at 50°C and 4°C. Additional studies to evaluate the decomposition mechanism are planned.

6. Adriamycin-DNA (Mr. Cradock)

Previous studies in our laboratory have demonstrated that the procedure utilized in manufacture of the adriamycin-DNA complex influences the biologic response. Specifically, the duration of autoclaving of DNA, rate of cooling, and electrolyte concentration were the major variables. This project was extended to evaluate additional parameters in mice and rats. The area under the plasma concentration-time curve in mice for the first four hours after IV administration of adriamycin-DNA complex in 0.1 M NaCl in mice was about four fold and 14 fold greater, respectively, than the complex prepared in water and free adriamycin. Similar differences between formulations were noted in anesthetized rats. Tissue Cxt values for the complex in 0.1 M sodium chloride were one-half in heart and double the values observed for free adriamycin in liver. No differences between formulations were observed in kidney and spleen. The affect of formulation in biliary excretion was evaluated in the rat since it is a principal route of elimination for anthracycline antibiotics. Initially, excretion was delayed in the complex treated group, but no differences were observed from 2-6 hours after drug administration.

The effect of complexation with DNA on the disposition and excretion appears to most substantially affect plasma clearance of adriamycin. Other affects including tissue Cxt levels in mice, and excretion appear to be modest and of short duration.

Additional studies are in progress to improve the adriamycin-DNA complex by utilizing alternate methods of sterilization. Also, pilot studies are underway to evaluate the effect of DNA on the disposition and activity of ellipticine. As with adriamycin, ellipticine exhibits an organ specific toxicity that might be altered by complexation with DNA.

Several other projects were investigated during the past year. These studies involved routine formulation, solubility and stability evaluations of several new agents.

Publications:

1. Cradock, J. C., Kleinman, L. M. and Davignon, J. P.: Intrathecal injections--a review of some pharmaceutical factors. Bull. Parenteral Drug Assoc. 31:237-248, 1977.

2. Rahman, A., Cradock, J. C. and Davignon, J. P.: Dissolution and absorption of the antineoplastic agent ellipticine. J. Pharm. Sci. 67:611-614, 1978.

3. Cradock, J. C., Kleinman, L. M. and Rahman, A.: Evaluation of some pharmaceutical aspects of intrathecal methotrexate sodium, cytarabine and hydrocortisone sodium succinate. Am. J. Hosp. Pharm. 35:402-406, 1978.

4. Flora, K. P., Cradock, J. C. and Ames, M. M.: A simple method for the estimation of amygdalin in urine. Res. Comm. Chem. Path. and Pharmacol. In Press.

Additional publications coauthored by members of the Analytical and Product Development Section are listed in the Pharmaceutical Resources Branch Report.

SUMMARY REPORT OF THE LABORATORY OF MEDICINAL CHEMISTRY AND BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1977 - September 30, 1978

The Laboratory of Medicinal Chemistry and Biology is an organizational entity resulting from the consolidation in 1975 of previously established sections from within the Drug Development and Drug Evaluation Branches of the Drug Research and Development Program, DCT, The Laboratory of Chemical Pharmacology, Experimental Therapeutics Program, DCT; and the Laboratory of Biochemistry, DCBD. The aim in establishing the new Laboratory was to bring about a closer relationship between medicinal chemists involved in the design and synthesis of new antitumor agents, and biologists involved in the elucidation of their biochemical pharmacology and in their clinical evaluation. It was felt in proposing this new organizational structure that knowledge of the molecular biology and etiology of cancer is approaching the level at which the biologist can now make useful early contributions to the rational design of new antitumor agents, rather than being restricted almost entirely to biochemical, pharmacological and clinical studies on agents whose antineoplastic activity has already been established on an empirical basis. While it is recognized that it would be premature and over-optimistic to propose that empirically-based screening approaches can in the foreseeable future be abandoned in favor of specific, rational antitumor drug design, the time now appears to have arrived when a mixture of empirical and rational approaches is warranted. Examples of drug classes in which opportunities for specific, target-directed design are now possible are agents affecting structure and function of tumor cell surface glycoproteins; inhibitors of viral oncogenesis; agents with specific or unique DNA-binding properties; transition-state inhibitors for key enzyme sites; and agents with specific tissue-localizing properties. In these and a number of other rapidly expanding research areas, the state of the art appears sufficiently advanced that the biologist with specific expertise can make contributions of value to the medicinal chemist in the design of new antitumor agents and in the modification of existing agents.

Conversely, from the point of view of the medicinal chemist, a closer interrelationship with the molecular biologist and the clinician offers the opportunity of obtaining specific biological assay data with the minimum of delay, in order to guide the course of further synthesis. In addition to providing routine antitumor screening, the biologist can design assay methods tailored to the specific target site under consideration. Assays for parameters such as inhibition of viral replication, immunostimulation, enzyme inhibition or tumor cell membrane binding can be provided where relevant to the activity under consideration. Thus, the juxtaposition within a single laboratory of medicinal chemists and biologists from a variety of different disciplines offers the prospect of accelerating drug development, and of supplementing the traditional empirical approaches which have yielded most of our clinically successful antitumor drugs up to the present time with specific target-site directed drug design programs.

The major organizational entities within the Laboratory of Medicinal Chemistry and Biology, in addition to the Drug Design and Chemistry Section, are the Molecular Biology and Methods Development Section, with the capacity to assess the effects of new agents on cell growth and cell cycle progression, and including a membrane biology group, with a specific interest in the role of tumor cell membrane alterations in drug resistance; and a newly established Applied Pharmacology Section which is concerned both with the mode of action of new agents at the molecular level and with disposition and metabolic studies of such agents in experimental animals and at the Phase I level. Particular stress is given to studies of agents originating from within the LMCB drug synthesis program. Some of the individual research projects presently under way in the Laboratory are summarized below.

This laboratory has had a long-term major interest in nucleosides related to the compound 5-azacytidine, an agent of proven value in the treatment of acute myelocytic leukemia. In previous reports, we described the synthesis and biological properties of the analog dihydro-5-azacytidine, a compound with significant activity in the L1210 leukemia test system. These studies have since been extended to the synthesis and biological testing of several related compounds with potential aneitumor activity. Among the most interesting of these is the arabinosyl analog of 5-azacytidine. As a result of the observed clinical activity of 5-azacytidine (5-AC), the arabinosyl derivative was suggested a number of years ago as an analogue of both 5-AC and cytosine arabinoside. However, a report by R. K. Robins in 1970, which described unsuccessful synthetic attempts to prepare the arabinoside, demonstrated that a straight-forward synthetic scheme would not secure this interesting nucleoside. We have now found that starting with the known 2,3,5-tri-O-benzyl-1-β-D-arabinofuranosyl-5-azacytosine, catalytic hydrogenation gives 5,6-dihydro-1-β-D-arabinofuranosyl-5-azacytosine. By using a novel oxidation procedure wherein the pertrimethylsilyl derivative undergoes a thermal elimination, the arabinoside (NSC 281272) has been synthesized in good yield. During the past year, a substantial amount of data has been collected on the pharmacological activity of arabinofuranosyl-5-azacytosine (ara-5-AC). In a direct comparison study with 5-azacytidine (5-AC) using the L1210 system, QD 1-9, ara-5-AC gave 147, 144 and 170 %ILS while 5-AC gave 134, 126 and 194 %ILS in respective controls. In comparison to an optimal dose of 3 mg/kg for 5-AC, the optimal dose of ara-5-AC was about 50 mg/kg. In good agreement with in vivo testing, the cytotoxicity of 5-AC against cultured L1210 cells was found to be about a log unit more potent than ara-5-AC. An L1210 subline resistant to 5-AC was also not sensitive to ara-5-AC in an in vivo assay. Similarly, cross-resistance of ara-5-AC with cytosine arabinoside was also demonstrated in vivo with an appropriate L1210 subline. Biochemical studies to determine the kinases utilized by ara-5-AC in its conversion to nucleotide forms, and also to determine whether the compound, like araC and 5-AC, is deaminated by cytidine deaminase, are presently being initiated.

Ara-5-AC was synthesized by a novel silylation-mediated oxidation of the corresponding, dihydro nucleoside. This unusual reaction was discovered during the course of an analytical trimethylsilylation of 5,6-dihydro-5-azacytidine. This reaction may be of general usefulness in nucleoside chemistry, since previous attempts by several laboratories to prepare ara-5-AC by more conventional routes had been consistently unsuccessful. The kinetics mechanism, scope and synthetic utility of this new reaction are presently being investigated. Present studies indicate that for 5,6-dihydro-5-azacytosine, the arabinofuranosyl nucleoside is oxidized at only one-fourth the rate for the ribofuranosyl nucleoside. Dihydropyrimidines were dehydrogenated at substantially slower rates than dihydro-S-triazines.

Synthesis of a number of transition-state enzyme inhibitors of potential usefulness in cancer chemotherapy is presently being attempted. Among these are (1) phosphonacetyl-L-glutamine, a potential inhibitor of glutamine-dependent carbamyl phosphate synthetase, the first enzyme in the pathway of de novo pyrimidine biosynthesis; and (2) a potential cytidine deaminase inhibitor incorporating as the pyrimidine analogue moiety, the unusual seven-membered ring of the purine deaminase inhibitor, 2'-deoxycoformycin.

Other synthetic projects currently in progress are modifications of natural products which have known antitumor activity, but which have not achieved clinical status, either because of low activity, or because of undesirable pharmacological or toxicological properties. Among these agents is colchicine: regression analysis of 15 colchicine derivatives gave a good correlation of biological activity with partition coefficient (log P) and with substitution on the nitrogen atom. While maintaining proposed derivatives within the ideal log P range of 1.5 to 1.9, various N-substituted analogues are being synthesized. Another such agent is chartreusin. This compound is a fermentation product that achieved DN2A status in the program on the basis of its L1210, P388 and B16 activity. Subsequently, it was found to be rapidly excreted in bile and also difficult to formulate because of poor water solubility. A synthesis project has been initiated for the purpose of replacing the unusual disaccharide moiety of chartreusin with the readily available disaccharide, D-maltose. The resulting derivative might be expected to have a greater water solubility because of the increased number of hydroxyl groups in the molecule. The structure of the proposed synthetic derivative, however, has many similarities with the parent (including the α-disaccharide linkage). Unless the unusual disaccharide portion of chartreusin plays a crucial role in its antitumor activity, the synthetic derivative may, it is hoped, retain the desirable biological properties of the parent while lacking its undesirable bilary excretion properties.

This Laboratory has pursued an active interest in the synthesis of organ-specific antitumor agents based on modification of chemical structures from other areas of pharmacology which are known to localize in specific tissues. This program has led to the synthesis of spirohydantoin mustard (NSC 172112) and the diaminodiaziridinylbenzoquinone derivative AZQ (NSC 182986), both of which have been selected for DN2 status as potential CNS antitumor agents and which are now undergoing pharmacological and toxicological study prior to clinical trial. Another such agent is propranolol mustard, synthesized because of the known ability of propranolol to localize in lung tissue. A selective chlorination of a triol intermediate using tosyl chloride in DMF solution gave propranolol mustard in moderate yield. This compound gave 46% ILS in the P388 test system and has been scheduled for testing against Lewis lung carcinoma.

Another major interest of this Laboratory is the development of quantitative structure:activity correlations in the antitumor drug area. These studies are carried out both within our own laboratories and via a contract with Prof. Corwin Hansch of Pomona College. Computer techniques are used to analyze enzyme inhibition and in vivo screening data. These data are then correlated with molecular structural parameters using computerized regression analysis techniques. The potential use of such correlations is to generate leads for new synthesis, to increase the rationale for selection of new compounds to be tested, and to reduce the number of synthetic analogs necessary to obtain the optimally active member of a new series. Contract-supported structure:activity studies have now been completed on the terephthalanilides, aniline mustards, adriamycin analogues, and phenyltriazenes; new studies are now underway on TEPA analogs, cis-platinum analogs, and the Cain acridines. Intramurally, structure-activity studies are presently underway in the colchicine and podophyllotoxin series.

A recent development of great interest to this laboratory has been the development within the pharmaceutical industry of several classes of potent inhibitors of the mammalian enzyme adenosine deaminase. A large number of presently available adenosine analogs exhibit antitumor, antiviral and antibacterial activity in in vitro model systems, but their usefulness in vivo has been limited by rapid enzymic deamination and consequent inactivation. The co-administration of an effective adenosine deaminase inhibitor would offer the possibility of preventing this inactivation and thus of potentiating the activity of these analogs. Two years ago, studies were initiated within this laboratory on the combination of the adenosine deaminase inhibitors 2'-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) with a number of adenosine analogues; this project has been carried out jointly with the Laboratory of Chemical Pharmacology. Against P388 cells in vitro significant potentiation by 2'-deoxycoformycin of the cytotoxic activity of seven such adenosine analogues was observed; these observations have since been extended to in vivo test systems with two of these analogs: cordycepin (3'-deoxyadenosine) and 9-β-xylofuranosyladenine. In vivo studies with the remaining analogues will be carried out when adequate supplies become available.

In addition an enzyme inhibition tritration assay method has been developed to measure levels of 2'-deoxycoformycin in biological fluids at drug concentrations as low as 0.2 nanomolar. Using this assay method, extensive pharmacological studies with 2'-deoxycoformycin have been carried out in the dog, in monkeys, and in man; the latter studies with human subjects were carried out in collaboration with Dr. John Smythe of the Royal Marsden Hospital, London, England. Unusual features of the pharmacology of the drug are its extremely long persistence in most tissues and the apparent absence of quantitatively significant metabolic conversion; the latter observation was surprising in view of the chemical instability of the compound. In rhesus monkeys, studies have been carried out in animals fitted with indwelling CSF catheters and given the drug both by intravenous and by intrathecal routes. These studies will be continued using 2'-dCF in combination with adenosylarabinoside in hopes of defining optimal doses and routes of administration for delivering antitumor and antiviral activity to the CNS with minimal systemic toxicity. Potential clinical uses include the treatment of herpes encephalitis and of leukemic and carcinomatous meningitis. In human subjects, the pharmacokinetics of 2'-dCF were similar to those noted in the dog and monkey; the major toxic effect was a marked lymphocytopenia seen in all patients given the drug at doses of 0.1 and 0.25 mg/kg on a daily x 5 schedule. These data will be used in supporting the introduction of 2'-dCF: araAMP combinations into clinical trials.

Another aspect of our ongoing studies with 2'-deoxycoformycin is the elucidation of the effects of this agent on immune function in experimental animals and also in human peripheral blood lymphocytes. Previous investigators have shown that the enzyme, adenosine deaminase, appears to be important for normal immunologic function. Lymphocytes responding to antigenic stimulation have high specific activity of the enzyme, and lymphocytes from patients born with a deficiency of the enzyme fail to perform normally in vivo or in vitro Normal lymphocytes can be made to behave abnormally in vitro as though they were ADA deficient by incubating them in the presence of inhibitors of ADA and excess adenosine or 2'-deoxyadenosine.

In this laboratory, significant inhibition by 2'-dCF of a number of indices of immune response have been noted. These include inhibition of the blast transformation of human blood lymphocytes by the T-cell mitogens phytohemagglutinin, concanavalin A and pokeweed mitogen; inhibition of the delayed hypersensitivity response in guinea pigs sensitized with PPD; and inhibition of the allograft rejection reaction in mice. These findings together with additional evidence of the lympholytic properties of 2'-dCF in humans support the importance of adenosine deaminase for some aspects of normal murine functioning and suggest that pharmacologic inhibition of adenosine deaminiase activity can be both a useful model for the study of the genetically induced disease, and an effective therapeutic immunosuppressive strategy.

It has long been known that adenosine deaminase exists in mammalian tissues in both small and large molecular weight forms. The major form of adenosine deaminase found in spleen, stomach, intestine and erythrocytes is that of the small molecular weight variant, while lung, liver and kidney contain the large form. In addition, mitogen stimulated lymphocytes and neoplasms of the lung and liver contain only the small forms of adenosine deaminase. In preliminary studies with the small and large forms of the enzyme from calf intestine, we have observed that the large form of the enzyme is much less sensitive to inhibition by 2'-dCF than is the small form. These studies are being continued and the pharmacological implications are being explored.

At the molecular level, the laboratory has been intensively pursuing basic studies on the mode of action of the antitumor and antiviral agents cordycepin (3'-deoxyadenosine) and xylosyladenine, in view of our earlier observation that the action of these agents in vivo was markedly potentiated by the adenosine deaminase inhibitor 2'-dCF. Measurement of the synthesis of L1210 cell nuclear rRNA, non-poly(A) hrRNA and poly(A) hrRNA revealed potentiation by 2'-deoxycoformycin of the inhibitory effect of cordycepin on all species of RNA, as well as poly(A) synthesis. No differences were noted in the size of nuclear poly(A) obtained from cells treated with cordycepin either in the presence or absence of the adenosine deaminase inhibitor. These results suggest that the potentiation by 2'-deoxycoformycin of the cytotoxic and antitumor effects of cordycepin on L1210 cells in vivo is related to inhibition of nuclear RNA synthesis. The effect of the adenosine deaminase inhibitor, 2'-deoxycoformycin (dCF), on the inhibitory effect of 9-β-D-xylofuranosyladenine (XA) on nuclear RNA synthesis was also examined. Fractionation of nuclear RNA into rRNA, non-poly(A)hrRNA and poly(A)hrRNA revealed the latter species of RNA to be less sensitive to XA in the absence of dCF; however, in the presence of dCF, all three species of nuclear RNA showed similar sensitivities. Nuclear poly(A) synthesis was among the most sensitive RNA fractions to XA, and was also inhibited to a greater degree by pretreatment of cells with dCF. These results indicate that XA is markedly potentiated by inhibition of adenosine deaminase, and that deamination serves as a major catabolic route for this drug. Studies on the action of these two agents are continuing, and have recently been extended to an investigation of their effects on base and 2'-O-methylation of nuclear RNA. Detailed analysis of RNA and synthesis and methylation by these procedures appears to be a sensitive and discriminating means of assessing the pharmacological action of nucleoside anticancer drugs. Further studies with agents such as 5-azacytidine and dihydro-5-azacytidine (synthesized in LMCB) will be undertaken to define their sites of action on the synthesis and processing of nuclear RNA.

In view of recent reports of enhanced antitumor activity of 5-fluorouracil when combined with thymidine, a project has been initiated on the effects of 5-fluorouracil on RNA synthesis and function, with particular emphasis on assessment of the effects of the drug on the synthesis, post-transcriptional processing and coding properties of messenger RNA. Poly(A)RNA from polyribosomes of regenerating rat liver from animals treated with 5-FU has been isolated and translated in a cell-free system prepared from wheat germ embryos. The products were analyzed by SDS-polyacrylamide gel electrophoresis, isoelectric focusing, and fluorography. Poly(A)$^+$RNA from 5-FU-treated rats (20 mg/kg) stimulated translation by 240 percent in comparison to poly(A)$^+$RNA from control animals. The stimulatory effect was associated with an overall increase in the quantity of products translated, as well as with a shift in the size of the proteins. No new translation products were observed.

In further studies, the synthesis of polysomal RNA after 5-FU treatment, and the incorporation of [^{3}H]5-FU into RNA was measured. Agarose-urea gel electrophoresis showed that poly(A)RNA labeled with [^{3}H]5-FU had a modal distribution in size of 21S and similar to that of control poly(A)RNA labeled with [^{3}H]orotic acid. rRNA labeled with [^{3}H]5-FU showed 45S, 28S and 18S RNA with an additional species at 38S in comparison to control rRNA. DEAE Sephadex-urea chromatography of alkaline hydrolysates of [^{3}H]5-FU-labeled rRNA indicated a greater distribution of label in the dinucleotide fraction (20%) vs that found in control rRNA (41%). Dihydroxyboronylphenylaminoethyl cellulose chromatography of [^{3}H]5-FU-labeled poly(A) indicated that 5-FU was present in internucleotide linkage and not in the 5'cap. These studies of the effects of 5-FU on RNA synthesis and function will shortly be extended to studies of the effects of the synergistic regimen of 5-FU plus thymidine.

Studies are continuing on the mode of action of the ansa macrolide maytansine, a plant product which was isolated and characterized by Kupchan and his coworkers in 1972, and which is now in Phase I/II clinical trial. In studies recently completed in this laboratory, maytansine was found to impair microtubule formation in mammalian cells and to produce metaphase arrest similar to that seen with the Vinca alkaloids. If the antitumor activity of maytansine is a consequence of its action on microtubular protein, there could well be a correlation between the tubulin-binding capacity and antitumor activity of compounds of the maytansine group. In order to test this hypothesis, four related maytansinoids (maysine, normaysine, maysenine and maytanvaline) were studied. Affinity for the Vinca binding site was assessed by the ability of the maytansinoids to compete with tritium-labeled vincristine for binding. Good correlation was observed, with maytansine and maytanvaline showing the greatest affinity for tubulin and also the greatest antitumor activity among the compounds tested. It was further concluded that the ester side-chain at C-3 is important both for antitumor activity and for the ability to compete with vincristine for binding to tubulin.

Active work has continued on the development of procedures to potentiate the activity and selectivity of antitumor agents by means of pharmacological and nutritional intervention. Of particular promise in the latter area have been our recent studies on the relationship between the membrane transport of amino acids and the cytotoxicity of the clinically important antitumor agent melphalan (phenylalanine mustard; L-PAM). Melphalan cytotoxicity to murine L1210 leukemia cells in culture is reduced in growth medium containing amino acids. Clonal growth studies indicated that the L-isomers of leucine and glutamine are principally responsible for reducing cytotoxicity. This decrease in cytotoxicity is accompanied by a corresponding decrease in melphalan transport into leucine protected cells. Kinetic analysis of the leucine-melphalan interaction indicates that although melphalan and leucine share a common transport system their affinities differ (leucine K_m=5-10 µM; melphalan K_m=35-50 µM). These results indicate that melphalan cytotoxicity is dependent upon transport by a high-affinity, carrier-mediated, leucine-preferring amino acid transport system. In recent attempts to extend these observations to in vivo systems, it has been found that co-administration of the glutamine-depleting enzyme glutaminase:asparaginase to tumor-bearing mice results in marked potentiation of melphalan activity, with a high percentage of long-term (>60 day) survivors being observed.

A long-term interest of this laboratory has been the pharmacology of the folate antagonists, and in particular, of the widely used agent methotrexate. Recently, we demonstrated the presence in the urine of human subjects and of monkeys which had received high-dose MTX of a metabolite, 7-hydroxyMTX, which is not seen after the administration of MTX at conventional dose levels. Although 7-hydroxymethotrexate is some two orders of magnitude less effective as an inhibitor of dihydrofolate reductase than MTX and does not contribute significantly to the folate antagonist activity of MTX, it may be of significant renal toxicological importance because of its limited aqueous solubility. The presence of this metabolite has now been confirmed by several other laboratories interested in the clinical pharmacology of high-dose MTX.

More recently, we have obtained unequivocal evidence by means of NMR studies and field desorption mass spectometry as to the structure of the 7-hydroxy metabolite. We have also supplied reference samples of 7-hydroxyMTX to a number of clinical pharmacology laboratories in the United States and abroad.

Efforts have continued on the development of techniques for the analysis of drug effects on proliferative cell cycle progression and for the application of such techniques to the characterization of cancer chemotherapeutic drugs and to the identification of new agents with potential therapeutic activity. These studies are not restricted to agents under development by LMCB, but encompass compounds being developed by the Developmental Therapeutics Program as a whole. The methods employed in this project are mammalian cell culture techniques as applied to the growth of tumor cells in suspension culture; flow microfluorometric determination of cell DNA content of fluorescent-stained cells; and processing, analyzing and plotting of cell DNA content frequency distributions by computer. During the current year, 18 drugs of interest were evaluated for their effects on L1210 cell cycle progression: dibromomannitol (NSC-94100), dibromogalactitol (NSC-104800), prospidine (NSC-166100), dianhydrogalactitol (NSC-132313), indicine-N-oxide (NSC-132319), gallium nitrate (NSC-15200), hexamethylmelamine (NSC-13875), neocarzinostatin (NSC-157365), anguidine (NSC-141537), bruceantin (NSC-165563), pyrazolo imidazole (NSC-51143), PCNU (NSC-95466), dihydro-5-azacytidine (NSC-264800), nogalamycin (NSC-70845), 7-O-CH_3-nogarol (NSC-293863), 7-O-CH_3-nogalarol (NSC-293862) and nogamycin (NSC-268450).

In view of the lack of chemotherapeutic agents effective in the treatment of malignant melanoma, a number of melanoma lines of both human and murine origin have recently been established in tissue culture, both for the purpose of screening for agents which may have activity against melanoma, and also in order to determine whether melanoma lines have specific nutritional requirements in terms of hormones and other growth factors. In initial studies, the most actively cytotoxic compounds detected to date against both melanotic (tyrosinase-positive) and amelanotic (tyrosinase-negative) lines have been 3-isopropylcatechol and p-butoxyphenol. These studies are continuing, utilizing test compounds from within the LMCB synthesis program and from the DTP drug development program as a whole.

The preceding report summarizes the objectives of the new Laboratory of Medicinal Chemistry and Biology, and outlines some of the research carried out within the Laboratory during the year. The individual project reports which follow describe this research in greater detail, and list the publications which have resulted from these studies.

Publications of the Laboratory of Medicinal Chemistry and Biology, 1977-78.

1. Beisler, J.A., Abbasi, M.M. and Driscoll, J.S.: The synthesis and antitumor activity of arabinosyl-5-azacytosine. Biochem. Pharmacol., 26: 2469-2472, 1977.

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The following publications describing collaborative studies with other laboratories also appeared during the period October 1, 1977 - September 30, 1978.

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IAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06144-01 LMCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Tyrosinase Activated Cytotoxic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.R. Breitman	Research Chemist	LMCB	NCI
OTHER:	M. Rabinovitz, Ph.D.	Research Chemist	LMCB	NCI
	J.S. Driscoll, Ph.D.	Head, Drug Design and Chem. Section	LMCB	NCI
	B. Keene	Microbiologist	LMCB	NCI

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.6	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A screen for agents specific against melanoma has been developed. Compounds have been selected for testing that are likely to be activated by tyrosinase, an enzyme unique for melanotic melanocytes. The screen tests agents for cytotoxicity against melanotic (tyrosinase positive) and amelanotic (tyrosinase negative) melanoma cells in culture.

Project Description:

General Objectives:

To find or synthesize compounds that can be converted by tyrosinase, an enzyme unique for melanotic melanocytes, to cytotoxic agents.

Major Findings:

Approximately 20 compounds have been tested in an in vitro screen, comparing the relative cytotoxicity of compounds against melanotic (tyrosinase positive) cells and amelanotic (tyrosinase deficient) cells. Two very active compounds were 3-isopropylcatechol and p-butoxyphenol which killed 50% of the cells at concentrations of approximately 3×10^{-5}M. In contrast, two compounds reported to be active against melanoma cells, p-methoxyphenol and DOPA, gave similar killing at approximately 100-fold greater concentrations. Preliminary results indicate that melanotic and amelanotic cells are equally sensitive to these agents.

Studies on the enzyme tyrosinase were initiated. This enzyme hydroxylates tyrosine to DOPA and oxidizes DOPA to DOPA-quinone. DOPA is required as an activator for the hydroxylation reaction but we have now found that it also is a competitive inhibitor of tyrosine hydroxylation, $K_i = 3 \times 10^{-4}$M. The K_m for tyrosine is 4×10^{-4}M.

Significance to Biomedical Research and Proposed Course:

This approach for the development of specific anti-melanoma agents will be continued. A structure-activity study on a series of phenols and catechols is planned. In addition, studies on growth conditions that affect the level of tyrosinase are planned.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07102-03 LMCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Pharmacology of Maytansine, Dimethylbenzylrifampicin, and other Ansa Macrolides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mary K. Wolpert, Ph.D.	Pharmacologist	DEB, NCI
Other:	Edward Chong, Ph.D.	Visiting Fellow	LMCB, NCI
	David G. Johns, M.D.,Ph.D.	Chief	LMCB, NCI
	James York	Co-Step Summer Student	LMCB, NCI

COOPERATING UNITS (if any)

Drug Evaluation Branch, NCI

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to study the mode of action and the pharmacology of antitumor agents of the ansa macrolide class. Attention is presently focused on the plant product maytansine and its naturally occurring and semi-synthetic derivatives. Maytansine was found to produce metaphase arrest in a manner similar to that seen with the Vinca alkaloids, and to impair microtubule formation in L1210 cells in culture. Topics of current interest are a) a comparison of the tubulin binding properties of maytansine with those of its structural analogs; b) bioassay methods for maytansine; and c) direct comparative studies of maytansine and vincristine with reference to cell uptake, cell kill, mitotic arrest, and physiological distribution.

The plant product maytansine is now undergoing Phase I and II clinical trial. We are studying maytansine at the subcellular (tubulin), cellular, and whole animal levels in an attempt to better understand its mode of action. Many of our studies are being conducted in direct comparison with vincristine. Although these two agents represent different chemical classes, many of their pharmacological properties are similar. It is hoped that these studies will result in findings with direct clinical application.

TUBULIN STUDIES:

One of our objectives was to determine the tubulin binding properties of several maytansinoids and attempt to correlate their binding activity with their *in vivo* antitumor activity. Of the approximately 20 known maytansinoids, we were able to obtain only the following four due to supply problems: maysine, normaysine, maysanine and maytanvaline. For these studies we took advantage of our earlier finding that maytansine and vincristine share a common tubulin binding site. Tubulin was prepared from rat brains using 2 cycles of polymerization and deploymerization according to the method of Shelanski and co-workers. A competitive binding assay using Whatman DE-81 paper discs as described by Wolff and co-workers was developed. The binding of ^{3}H-vincristine to tubulin was determined to 10 mM sodium phosphate buffer (pH 6.8), 10 mM MgC/2, 0.1 mM GTP, and in the presence or absence of a maytansinoid. Over a concentration range of 0 to 0.7 μM, ^{3}H-vincristine exhibited one binding site for tubulin and a binding constant (KA) of 11.43 x 10^6 M^{-1}. Using Dixon plots, we found that maytansine competitively inhibited vincristine binding with a Ki of 0.35 μM. Maytanvaline, which has an isopropyl group in place of the terminal methyl groups of C-3, was a competitive inhibitor with a Ki of 0.95 μM. Of the maytansinoids lacking the ester chain off C-3, normaysine was a competitive inhibitor with a Ki of 31.76 μM. No competition could be detected with maysine at concentrations as high as 20 μM. The Ki for maysenine was 4.83 μM, but the inhibition resembled that of a partial non-competitive inhibitor. Since only maytansine and maytanvaline had significant antitumor activity in mice bearing the P388 leukemia, it was concluded that the ester side-chain off C-3 is important for both *in vivo* antitumor activity and the ability to compete with vincristine for binding to tubulin. The basis for the different type of inhibition obtained with maysenine, which lacks both the ester side-chain and the epoxide group, is not known. Perhaps in the absence of an ester side-chain, the epoxide group interferes with the binding of a maytansinoid to tubulin. The finding that maysenine is more potent in competing for the vincristine-^{3}H binding site than either maysine or normaysine is in agreement with this hypothesis. These results were presented at the 1978 Annual Meeting of the American Association for Cancer Research (Proc. AACR, Vol.19, 110, 1978) and are presently being prepared for publication.

CELLULAR STUDIES:

Comparative studies of maytansine and vincristine were undertaken in L1210 cells in culture to better understand the pharmacology of both agents. Both drugs were directly compared at several equimolar concentrations to determine their effects on cell growth, cell viability by the dilute agar cloning assay and mitotic index. In all three types of assay, maytansine was more potent than vincristine. In order to determine if the difference in potency could be partially explained by a difference in uptake and/or retention of the two agents, uptake studies were initiated. Preliminary work indicated that about twice as much ^{3}H-maytansine as ^{3}H-vincristine was accumulated by L1210 cells over 60 min. Retention studies are planned. In P388 cells with acquired resistance to vincristine and with cross-resistance to maytansine, essentially no uptake was detected after 60 min at concentrations as high as 1×10^{-6} M.

PHYSIOLOGICAL DISTRIBUTION:

As a follow-up to our earlier work in which we demonstrated that maytansine was more potent *in vivo* than vincristine in producing mitotic arrest in both L1210 and P388 ascites cells, a comparative study of the physiological distribution of both agents in mice was undertaken to look for qualitative and quantitative differences. Plasma and several tissues, including the sciatic nerves, were examined for radioactivity at 24, 48 and 72 hrs after a single optimal therapeutic dose (4 mg/kg for vincristine). Tissue levels were found to persist in the thymus, lymph nodes, and pancreas, but to decline rapidly from the plasma, liver and other major organs. Brain and sciatic nerves contained barely detectable drug levels. Similar studies are planned for maytansine whenever the ^{3}H-labeled material becomes available.

PUBLICATIONS:

Johns, D. G., Wolpert-DeFilippes, M. K., Mandelbaun-Shavit, F., Chabner, B. A. Bono, Jr., V. H., and Adamson, R. H.: Pharmacological action of maytansine. *Current Chemotherapy* II: 1180-1182, American Society for Microbiology, 1978.

Johnson, R. K., Inouye, T. and Wolpert-DeFilippes, M. K.: Stathmokinetic and therapeutic effects of maytansine in mice bearing P388 and L1210 leukemias. *Biochemical Pharmacology*, (in press) 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07103-03 LMCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Studies on the Clinical Pharmacology of the Folic Acid Antagonists

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Johns, M.D., Ph.D.	Chief	LMCB	NCI
Other:	Samuel A. Jacobs, M.D.	Montefiore Hosp. & Univ. of Pittsburgh		
	Michael Przybylski, Ph.D.	Visiting Fellow	LCP	NCI
	Herman Yeh, Ph.D.	Sr. Investigator	LC	NIAMDD

COOPERATING UNITS (if any)
Laboratory of Chemical Pharmacology, DTP, NCI
Montefiore Hospital, University of Pittsburg, Pittsburgh, PA.
Laboratory of Chemistry, NIAMDD

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

While methotrexate has been in clinical use for a number of years as an antitumor agent, the recent introduction of high dose schedules followed by citrovorum factor rescue in the treatment of solid tumors has resulted in renewed interest in this agent, and has revealed that our knowledge of the clinical pharmacology of this and related folate antagonists is still incomplete. During the past year, unequivocal evidence has been obtained by field desorption mass spectrometry and by proton NMR spectrometry of the structure of the major metabolite of methotrexate, 7-hydroxymethotrexate.

In view of the great clinical interest in high-dose MTX therapy, studies have continued on the pharmacology of MTX and of its metabolite, 7-hydroxyMTX. Further lots of 7-hydroxyMTX have been prepared biologically, by isolation and purification from the urine of Rhesus monkeys given high-dose MTX by slow infusion. Numerous reference samples of 7-hydroxyMTX have been shipped in response to requests from extramural investigators with an interest in high-dose MTX therapy and clinical pharmacology.

Unequivocal evidence has been obtained by field desorption mass spectrometry of the structure of 7-hydroxyMTX. The field desorption mass spectra were recorded with a Varian MAT CH 711 double focusing spectrometer equipped with a dual e.i./f.d. ion source. Experimental conditions of the instrument were: 800 (10% valley-definition) resolution, 11 kV total potential difference between field emitter anode/cathode, $150^{\circ}C$ ion source temperature, 2.5 kV voltage of the electron multiplier used for ion registration. 7-HydroxyMTX and its parent drug MTX were found to be clearly characterized by their field desorption spectra. The spectrum of the metabolite showed a most abundent peak at m/e 471, corresponding to the protonated quasi-molecular ion MH^+, while MTX yielded an MH^+ ion at m/e 455. Moreover, a significant fragmentation pattern was found for both MTX and its hydroxylated metabolite. Fragments arising by cleavage of the N_{10}-CH_2 bond adjacent to the pteirdinyl moiety were found at m/e 176/177 for MTX and at m/e 193/194 for 7-hydroxyMTX. Identical fragments at m/e 281 appear in both spectra, due to the p-aminobenzoylglutamate residue of the molecule. These fragment ions provide direct evidence for the presence of the hydroxyl group solely in the 7-position of the pteridine ring.

Confirmatory evidence of the structure of the 7-hydroxy metabolite was obtained by proton magnetic resonance. Proton NMR spectra of MTX and 7-hydroxyMTX, dissolved in DMSO-d_6, were obtained on a JEOL FX-100 spectrometer with Fourier transform attachment. The metabolite was identified as 7-hydroxyMTX based on its ^1H spectrum which is identical to that of MTX except that the resonance at δ8.64 was not observed and the resonance corresponding to the C-9 protons was moved upfield to δ4.60 by 0.26 ppm. Since the resonance at δ8.64 found in MTX is assigned to the proton on C-7, absence of this signal in the metabolite indicates that hydroxylation must take place at the C-7 position. This is consistent with the upfield shift for protons on C-9 in 7-hydroxyMTX as a result of hydroxylation at the C-7 position.

PROPOSED COURSE:

Because of the current interest in high-dose aminopterin, studies will be carried out in Rhesus monkeys to determine whether this compound, like MTX, yields a 7-hydroxy metabolite when given in a high-dose regimen. The question has clinical relevance, since one of the reasons for substituting high-dose aminopterin for high-dose MTX is the prospect of avoiding the renal toxicity frequently seen as a consequence of high-dose therapy with the latter drug.

PUBLICATIONS:

Jacobs, S. A., Derr, C. J. and Johns, D. G.: Accumulation of methotrexate diglutamate in human liver during methotrexate therapy. Biochem. Pharmacol. 26: 2310-2313, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07105-03 LMCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biochemical and Pharmacological Studies of Pyrimidine Nucleoside Analogs with Antitumor Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mary K. Wolpert, Ph.D.	Pharmacologist	DEB	NCI
Other:	David G. Johns, M.D., Ph.D.	Chief	LMCB	NCI
	John S. Driscoll, Ph.D.	Chemist	LMCB	NCI

COOPERATING UNITS (if any)

Drug Evaluation Branch, NCI

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the pharmacologic activity and the biochemical mode of action of new antitumor agents of the pyrimidine nucleoside class. Present studies are focused on 5-azacytidine and 5,6-dihydro-5-azacytidine, a reduced analog of the latter with greater chemical stability than the parent compound. Topics of current interest are combination chemotherapy studies both in vitro and in vivo and studies designed to test whether the dihydro derivative is a pro-drug of the parent or acting in its own right.

PHS-6040
(Rev. 10-76)

5,6-Dihydro-5-azacytidine (DHAC), which is chemically more stable than 5-azacytidine (5-AC), was found to be an effective chemotherapeutic agent in mice bearing L1210 leukemia. However, it was found to be considerably less potent. An optimal dose for 5-AC is about 1-3 mg/kg ip on a QID, days 1-9 schedule and about 100-200 mg/kg for the derivative. Therefore, attempts were made to increase the therapeutic activity of DHAC by rational combination chemotherapeutic experiments. Attempts were also made to determine if DHAC was a pro-drug of 5-AC

COMBINATION CHEMOTHERAPY STUDIES:

Studies were designed to investigate the effects of 5-AC and DHAC on the growth of HeLa cells in culture either alone or in combination with other agents. Tetrahydrouridine (THU), a cytidine deaminase inhibitor, was found at 10^{-4} M to enhance the cytotoxicity of DHAC at both 10^{-5} M and 10^{-4} M, but not to affect the cytotoxicity of 5-AC. Based on these observations, the effect of DHAC + THU both administered either ip or orally, was determined against L1210 leukemia over a complete dose range from inactivity to toxicity. THU did not enhance the antitumor effectiveness of DHAC by either route of administration. Oral THU also did not enhance the antitumor effectiveness of oral 5-AC. Next it was observed that Thymidine at 10^{-4} M significantly increased the cytotoxicity of 5-AC but not DHAC to HeLa cells in culture. Therefore, the effect of thymidine at 500 mg/kg on an every 4th day treatment schedule was examined on the antitumor effectiveness of 5-AC on the same schedule in mice bearing L1210 leukemia. Again no enhancement was detected.

PRO-DRUG STUDIES:

Studies have recently been initiated with ^{3}H-DHAC to determine whether the dihydro compound is converted to AC in vivo and in leukemic cell suspensions. High pressure liquid chromatographic methods are being developed for the separation of DHAC, AC, and their respective metabolites in cell extracts. These studies are still in progress.

PUBLICATIONS:

Futterman, B., Derr, C. J., Beisler, J. A., Abbasi, M. M. and Voytek, P.: Studies on the cytostatic action, phosphorylation and deamination of 5-azacytidine and 5,6-dihydro-5-azacytidine in HeLa cells. Biochemical Pharmacology, 27: 907-911, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07106-03 LMCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Adenosine Deaminase Inhibitors as Potentiators of the Pharmacological Activity of Adenosine Analogs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Johns, M.D.,Ph.D.	Chief	LMCB	NCI
Other:	Marvin M. Chassin, M.D.	Clinical Assoc.	LMCB	NCI
	Richard H. Adamson	Chief	LCP	NCI
	David Poplack, M.D.	Sr. Investigator	POB	NCI

COOPERATING UNITS (if any)
Laboratory of Chemical Pharmacology, DTP, NCI
Pediatric Oncology Branch, COP, NCI

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A number of presently available adenosine analogs possess antitumor activity. Their usefulness has been limited by their rapid metabolism by adenosine deaminase. 2'-Deoxycoformycin, a powerful inhibitor of adenosine deaminase has been able to potentiate the antitumor activity of several adenosine analogs in vitro and in vivo. The purpose of this work was to study the pharmacokinetics of 2'-deoxycoformycin in monkeys with special attention to the distribution of the drug across the blood-brain barrier and to study the pharmacokinetics and toxicity of 2'-deoxycoformycin in man in conjunction with Phase I clinical trials presently under way.

PHS-6040
(Rev. 10-76)

Earlier studies from this and other laboratories have demonstrated that 2'-deoxycoformycin, an inhibitor of the enzyme adenosine deaminase potentiates the antitumor and antiviral effect of adenosine analogs which are metabolized by that enzyme.

In view of the potential usefulness of 2'-dCF in combination with adenosine analogs in antitumor and antiviral therapy, we have extended earlier studies on the pharmacokinetics and distribution of this compound in monkeys and man.

In collaboration with Dr. David Poplack, POB., NCI, Rhesus monkeys fitted with indwelling CSF catheters have been given 2'-dCF by IV and intrathecal routes. Plasma disappearance curves are similar to those obtained using foxhound dogs and the drug enters CSF with peak levels that are 10% of corresponding plasma levels. These studies will be continued using 2'-dCF in combination with adenosylarabinoside in hopes of defining optimal doses and routes of administration for delivering antitumor and antiviral actvity to the CNS with minimal systemic toxicity. Potential clinical uses include the treatment of herpes encephalitis and of leukemic and carcinomatous meningitis.

In collaboration with Dr. John Smyth of the Royal Marsden Hospital, England 2'-dCF has been given to human cancer patients.

Plasma drug disappearance curves demonstrated $\alpha t_{1/2}$ 37-69 minutes and $\beta t_{1/2}$ 165-156 minutes and an estimated $\gamma t_{1/2}$ of 5-11.5 hours. Twenty-five to 30% of administered drug was detected in the urine and urinary clearance was estimated at 31-71 ml/min. Doses of 2'-dCF of 0.1 mg/kg, and 0.25 mg/kg and 1.0 mg/kg inhibited the patient's lymphocyte adenosine deaminase to 20% of control activity. Marked lymphocytopenia was noted in all patients given 2'-dCF on a daily x 5 schedule.

This data will be used in supporting the introduction of 2'-dCF into clinical trials in this country.

PUBLICATIONS:

Adamson, R. H., Chassin, M. M., Chirigos, M. A. and Johns. D. G.: Mode of Action. Some aspects of the pharmacology of the adenosine deaminase inhibitors 2'-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl)-adenine. Current Chemotherapy II: 1116-1118, American Society for Microbiology, 1978.

Chassin, M. M., Adamson, R. H., and Johns, D. G.: An enzyme inhibition assay for 2'-deoxycoformycin and its application to the study of the relationship between drug concentration and tissue adenosine deaminase in the dog. Biochemical Pharmacology (in press), 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07107-03 LMCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biological Studies on Metastatic and Non-metastatic Melanomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. R. Breitman	Research Chemist	LMCB	NCI
OTHER:	J. Stoychkov	Guest Worker	LMCB	NCI
	B. Keene	Microbiologist	LMCB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

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NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Attempts are being made to grow human and murine melanoma cell lines in a completely defined medium. In the absence of serum, a growth response only to transferrin has been observed. No growth response to the other hormones and factors previously reported has been seen. A systematic examination of all components in the medium is underway to determine, as seems likely, what changes in the nutritional portion of the medium are responsible for the lack of normal response.

PHS-6040
(Rev. 10-76)

Project Description:

General Objectives:

To define the growth requirements of established human and animal melanoma cell lines with particular emphasis on defining the factors and hormones in the serum component of the medium.

Major Findings:

In the FY78 Annual Report it was reported that several human melanoma cell lines had a growth response to insulin, transferrin, progesterone, LRF, and NIH.FSH. The studies had been conducted at the University of California, San Diego. Attempts to repeat these findings at NIH have been unsuccessful. Because of the complex nature of tissue culture medium and the sensitivity of cells to medium in which serum is absent or is at a very low concentration, these negative results were not surprising. A systematic check of the components of the medium is now underway. At present, only a growth response to transferrin has been observed.

Significance to Biomedical Research and Proposed Course:

The clarification of what hormones and factors are required for the growth of melanoma cells should have great therapeutic value. These growth factors could be used e.g. as carriers of cytotoxic agents. It is planned that a greater effort will be devoted to this area of research in the coming year.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07108-02 LMCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Adenosine Deaminase Inhibitors as Immunosuppressive Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marvin M. Chassin, M.D.	Clinical Associate	LMCB	NCI
Other:	David G. Johns, M.D.,Ph.D.	Chief	LMCB	NCI
	Richard H. Adamson, Ph.D.	Chief	LCP	NCI
	Arthur C. Louie, M.D.	Clinical Associate	LMCB	NCI

COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacology, DTP, NCI

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NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of these studies is to examine the effect of 2'-deoxycoformycin and other potent inhibitors of adenosine deaminase on in vitro parameters of human peripheral blood mononuclear cell function, and on in vivo parameters of immune function in experimental animals.

PHS-6040
(Rev. 10-76)

The enzyme, adenosine deaminase (ADA), appears to be important for normal immunologic function. Lymphocytes responding to antigenic stimulation have high specific activity of the enzyme, and lymphocytes from patients born with a deficiency of the enzyme fail to perform normally *in vivo* or *in vitro*. Normal lymphocytes can be made to behave abnormally *in vitro* as though they were ADA deficient by incubating them in the presence of inhibitors of ADA and excess adenosine.

The purpose of the present studies is (1) to examine the effect of 2'-deoxycoformycin (2'-dCF), a potent inhibitor of ADA, on *in vitro* parameters of human peripheral blood lymphocyte function, (2) to examine the effects of 2'-dCF on *in vivo* parameters of immune function in animals and (3) to examine the adenosine deaminase activity of lymphocytes from patients with the T cell malignancy Sezary syndrome/mycosis fungoides.

Human blood lymphocytes were stimulated to undergo blast transformation by the T cell mitogens phytohemagglutinen, concanavalin A and pokeweed mitogen. This transformation was inhibited in a dose responsive manner by 2'-deoxycoformycin. The ID_{50} concentration was $\sim 10^{-6}$ M in all cases. In order to demonstrate inhibition there was a requirement for incubation of cells in 2'-dCF for 72^{o} before addition of mitogen. This requirement for preincubation is not evident for demonstrating potentiation of adenosine mediated inhibition of lymphocyte response. Similar results were obtained using murine splenic lymphocytes and the same mitogens.

Guinea pigs were sensitized to mycobacterium and their delayed hypersensitivity skin test response to challenge with PPD was measured. Treatment with 2'-dCF either before sensitization or before recall significantly diminished skin test response.

Monkeys were treated with 2'-dCF for a one week period. The response of lymphocytes from these animals, when tested *in vitro* for responsiveness to non-specific mitogen, was diminished compared to control animal cells and to their own cells when tested pre-treatment.

Mice were treated with 2'-dCF for 3-10 days. This produced a marked reduction in splenic size, which histologically represented loss of lymphocytes from both T and B cell areas. When the remaining splenic lymphoid cells were tested *in vitro* for response to non-specific mitogens they responded normally. However, natural killer activity of these cells was diminished, suggesting that cells able to perform this function are more susceptible to killing by 2'-dCF.

Peripheral blood lymphocytes from patients with the Sezary syndrome have been examined for adenosine deaminase activity. Whereas others have found that T-lymphoblasts from patients with acute lymphocytic leukemia have high ADA activity, we have found that Sezary T cells have ADA activity comparable to normal T cells and normal pooled peripheral lymphocytes. Thus these cells may be more susceptible to killing by 2'-dCF alone or by 2'-dCF in combination with adenosine analogs than ALL blasts.

These findings together with additional evidence of the lympholytic properties of 2'-dCF in humans obtained by others, support the importance of adenosine deaminase for some aspects of normal immune functioning and suggest that pharmacologic inhibition of adenosine deaminase activity can be both a useful model for the study of the genetically induced disease, and an effective therapeutic immunosuppressive strategy.

Applied Pharmacology Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1977 - September 30, 1978

The establishment of the Applied Pharmacology Section was officially approved in March, 1978. The objectives of this newly formed section are to conduct pharmacological studies of anticancer drugs under development by the Division of Cancer Treatment, with particular emphasis on agents arising from within LMCB.

At present, the Section is primarily engaged in biochemical and molecular biological studies of nucleoside anticancer agents. Two of the four projects described in this report deal with the effect of adenosine analogs on the synthesis and processing of nuclear RNA and the regulation of transcription of phosphorylation of nonhistone proteins. The potentiation of these analogs by the adenosine deaminase inhibitor, 2'-deoxycoformycin, is also an integral part of these projects. Potentiation of adenosine analogs by dCF may prove to be an important means of developing synergistic combinations of drugs without enhancing general cytotoxicity.

A third project deals with the effect of 5-fluorouracil on the translation of mRNA in a cell-free translation system. This aspect to the cytotoxic effects of this established anticancer drug is now receiving wide attention as a result of studies showing that the effects of 5-FU on RNA may contribute significantly to the antitumor effects of the drug.

The fourth project deals with isozymic variants of adenosine deaminase in normal and neoplastic tissues and the significance of the conversion of one form to another with respect to neoplasia. An integral part of this project is related to the sensitivity of the adenosine deaminase isozymes to adenosine deaminase inhibitors and the relationship of these effects to the inhibition of adenosine deaminase in various tissues. The latter aspect is particularly important for the establishment of the mechanism by which adenosine deaminase inhibitors, such as 2'-deoxycoformycin, exert their immunosuppressive effects.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06145-01 LM

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Characterization of Adenosine Deaminase in Normal and Neoplastic Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Joy Constine, M.D.	Research Associate	LMCB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Applied Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	-0-

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Purification and pharmacological criteria will be developed for studying the isoenzymes of adenosine deaminase in normal and neoplastic tissues. Gel filtration with Sephacryl C-200, ion exchange chromatography with QAE-Sephadex and immunochromatography with anti-adenosine deaminase IgG-Sepharose will be employed. Pharmacological criteria for differentiating between the different molecular species of adenosine deaminase will be developed with the inhibitors 2'-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl)adenine. The interconversion of the small molecular weight species to the large molecular weight enzyme via a conversion factor in liver will also be assessed.

OBJECTIVES:

Adenosine deaminase (ADA) (E.C. 3.5.4.4) catalyzes the irreversible hydrolytic deamination of adenosine to inosine and NH_3. ADA is of importance in the inactivation of antineoplastic adenosine analogs as demonstrated by the marked synergistic effect that adenosine deaminase inhibitors have on the chemotherapeutic activity of these drugs. In addition, the absence of ADA in lymphocytes and other tissues is associated, in part, with the clinical syndrome, severe combined immunodeficiency disease.

Multiple forms of adenosine deaminase have been reported in various mammalian tissues, as well as in other animal species. These major isozymes containing additional variants have been described with molecular weights varying from 33,000-47,000, 114,000-230,000 and 230,000-440,000, respectively. In addition there is some evidence that the small and large molecular weight forms are interconvertible via the action of a converting factor, although no differences in kinetic properties between the two forms of ADA have been found. The major form of ADA found in spleen, stomach, intestine and erythrocytes is that of the small molecular weight variant, while lung, liver and kidney contain the large form. Moreover, conversion factor is found in the latter tissues but not in the former. In addition, mitogen stimulated lymphocytes and neoplasms of the lung and liver contain only the small forms of ADA and no conversion factor.

Therefore, it is of importance to characterize the large and small molecular weight forms of ADA, and to determine their relative sensitivities to adenosine deaminase inhibitors. Normal rat liver and transplantable rat hepatoma 3924A will be used to purify the large and small forms of ADA, respectively. The conversion factor activity in normal liver will be measured utilizing the two purified forms of ADA. Pharmacological methods of discriminating between the large and small molecular weight forms of ADA will be assessed using the adenosine deaminase inhibitors, 2'-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl)adenosine (EHNA).

METHODS EMPLOYED:

Purification of ADA from the 100,000 x g supernatant fluid of rat liver and hepatoma 3924A by $(NH_4)_2SO_4$ precipitation, Sephacryl C-200 gel filtration, QAE-Sephadex chromatography and anti-ADA IgG-Sepharose; preparation of anti-ADA-IgG by immunization of rabbits with the large and small forms of calf intestine ADA prepared from commercial ADA after Sephacryl filtration; gel electrophoresis and isoelectric focusing of ADA to assess purity; assay of ADA spectrophotometrically to measure its activity and its inhibition by adenosine deaminase inhibitors.

MAJOR FINDINGS:

Calf intestine ADA was resolved into three species by Sephacryl gel filtration of molecular weights 38,000 (E_S), 57,000 (E_I) and a form greater than 200,000 (E_L) daltons. The sensitivities of the three forms of ADA to

2'-deoxycoformycin and EHNA was measured. The ID_{50} for 2'-deoxycoformycin for E_L, E_I and E_S was 2×10^{-8} M, 8×10^{-11} M and 2×10^{-11} M, respectively. The ID_{50} for EHNA for E_L, E_I, E_S was 1×10^{-7} M, 3×10^{-10} M and 2×10^{-11} M, respectively. Thus, forms E_S and E_I of ADA could easily be discriminated from form E_L on the basis of their respective sensitivities to 2'-deoxycoformycin and EHNA.

PROPOSED COURSE:

Purification of ADA from rat liver and hepatoma 3924A is being pursued. In addition, characterization of conversion factor from rat liver will be undertaken to understand its role in regulating the multiple forms of ADA and hence adenosine metabolism. Specific antibodies against the large and small forms of ADA and the conversion factor will be prepared in order to develop a radioimmunoassay for these macromolecules.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07109-02 LMCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effect of Anticancer Drugs on the Synthesis and Processing of Nuclear RNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Ann L. Peale	Biologist	LMCB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Applied Pharmacology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The synthesis and processing of nuclear ribosomal RNA (rRNA) and heterogeneous RNA (hnRNA) will be studied in vitro in L1210 leukemia cells. [^{3}H]Uridine will be used to pulse label and measure the synthesis of various species of nuclear RNA in L1210 cells. The methylation of rRNA and hnRNA, as well as the polyadenylation of hnRNA will be examined as representative post-transcriptional processes. The nucleoside anticancer agents, cordycepin (3'-deoxyadenosine) and xylosyladenine will be used to examine their specific inhibitory action on rRNA, poly(A), non-poly(A)hnRNA and poly(A)hnRNA synthesis in the presence and absence of the adenosine deaminase inhibitor, 2'-deoxycoformycin.

PHS-6040
(Rev. 10-76)

OBJECTIVES:

Many anticancer drugs act by inhibiting the synthesis of mRNA via interference with the activity of either RNA polymerases or the DNA template. Nucleolar RNA polymerase I and nucleoplasmic RNA polymerase II transcribe the 45S precursor to rRNA, and the heterogeneous RNA (hnRNA) precursor to mRNA, respectively. These two species of RNA undergo further modification or processing via 1) polyadenylation of the 3'-terminus and methylation of internal adenine as well as 5'-terminal guanine and ribose in hnRNA and 2) 2'-O-methylation of ribose in 45S rRNA. These unique modifications confer biochemical specificity for the nucleolytic trimming of the rRNA precursor, as well as recognition features for the transport of mRNA from the nucleus and its binding to the 40S ribosomal subunit. The objectives of this project are to characterize the inhibitory activity of anticancer agents, viz. adenosine analogs, on the synthesis and processing of rRNA and hnRNA. Analysis of the sensitivity of these biochemical processes to anticancer drugs and the synergistic action of adenosine deaminase inhibitors will allow an assessment of their role as biochemical targets for the design of new chemotherapeutic agents.

METHODS EMPLOYED:

Isolation of nuclei from L1210 cells by Triton X-100 and sucrose gradient centrifugation; incorporation of [^{3}H]uridine into mRNA from L1210 cells; incorporation of [^{3}H-methyl]-methionine into ribose and bases of nRNA; differential pH extraction of rRNA with SDS-sodium acetate-NaCl (pH 5.1) and phenol mixture (phenol:cresol:water, 7:2:2 v/v, containing 0.1% 8-hydroxyquinoline) and hnRNA with SDS-Tris-urea (pH 9.0) and phenol mixture; isolation of poly(A)hnRNA by poly(U)Sepharose affinity chromatography; isolation of [^{3}H-methyl] 5'-cap of hnRNA, methylated mononucleotides and 2'-O-methylated dinucleotides after alkaline hydrolysis by DEAE-Sephadex-urea chromatography and thin layer chromatography; agarose-urea gel electrophoresis of nuclear RNA and poly(A); assay of adenosine deaminase spectrophotometrically at 305 nm with formycin A as substrate.

MAJOR FINDINGS:

1. The effect of the adenosine deaminse inhibitor, 2'-deoxycoformycin, upon the inhibitory action of cordycepin on nuclear RNA synthesis was tested in regenerating rat liver 18 hr after partial hepatectomy. The 50% inhibitory effect (ID$_{50}$) of 2'-deoxycoformycin on adenosine deaminase activity was 6-7 μg/kg in both normal and regenerating liver. Cordycepin inhibited nuclear ribosomal RNA, non-poly(A)hnRNA and poly(A)hnRNA at ID$_{50}$ doses of 15, 18 and 17 mg/kg, respectively, 45 min after treatment. Pretreatment for 10 min with 2'-deoxycoformycin resulted in a 27-33% decrease in the ID$_{50}$ of cordycepin for all three species of nuclear RNA. Maximum potentiation by 2'-deoxycoformycin was achieved at 4 hr after cordycepin treatment for nuclear ribosomal RNA and non-poly(A)hnRNA and at 45 min for poly(A)hnRNA. These results suggest that

the deamination of cordycepin is not a highly significant factor influencing its pharmacological activity in regenerating liver.

2. The effect of the adenosine deaminase inhibitor, 2'-deoxycoformycin, on the inhibitory effect of cordycepin on nuclear RNA synthesis was examined in L1210 cells in vitro. The ID_{50} for the effect of deoxycoformycin on adenosine deaminase was 4×10^{-8} M, and 100% inhibition was achieved at 5×10^{-7} M. Pretreatment of cells for 30 min with 1×10^{-6} M deoxycoformycin resulted in a reduction of the ID_{50} for cordycepin from 2.5×10^{-4} M to 1.8×10^{-5} M, as assessed by the measurement of [^{3}H]uridine incorporation into total RNA. Measurement of the synthesis of nuclear rRNA, non-poly(A)hnRNA and poly(A)hnRNA revealed potentiation by 2'-deoxycoformycin of the inhibitory effect of cordycepin on all species of RNA, as well as poly(A) synthesis. No differences were noted in the size of nuclear poly(A) obtained from cells treated with cordycepin either in the presence or absence of the adenosine deaminase inhibitor. These results suggest that the potentiation by 2'-deoxycoformycin of the cytotoxic and antitumor effects of cordycepin on L1210 cells in vivo is related to inhibition of nuclear RNA synthesis.

3. The effect of the adenosine deaminase inhibitor, 2'-deoxycoformycin (dCF), on the inhibitory effect of 9-β-D-xylofuranosyladenine (XA) on nuclear RNA synthesis was examined in L1210 cells in vitro. Pretreatment of cells for 15 min with a 100% inhibitory dose (1×10^{-6} M) of dCF resulted in approximately a 3-8-fold reduction in the 50% inhibitory dose (ID_{50}) of XA for [^{3}H]uridine and [^{3}H]thymidine incorporation into RNA and DNA, respectively. The ID_{50} for XA for RNA synthesis vs. DNA synthesis was 5-fold lower in the absence of dCF and 20-fold lower in the presence of dCF indicating the greater sensitivity of RNA synthesis to this inhibitor. Fractionation of nuclear RNA into rRNA, non-poly(A)hnRNA and poly(A)hnRNA revealed the latter species of RNA to be less sensitive to XA in the absence of dCF; however, in the presence of dCF, all three species of nuclear RNA showed similar sensitivities. Nuclear poly(A) synthesis was among the most sensitive RNA fractions to XA, and was also inhibited to a greater degree by pretreatment of cells with dCF. These results indicate that XA is markedly potentiated by inhibition of adenosine deaminase, and that deamination serves as a major catabolic route for this drug.

4. Cordycepin and XA inhibited methylation of nuclear RNA to a greater extent than RNA synthesis in L1210 cells in vitro. Inhibition of base methylation, 2'-O-methylation and 5'-cap methylation was equal to, 2 to 5-fold greater and 1.5-fold greater, respectively, than inhibition of RNA synthesis. Cordycepin was more potent than XA in inhibiting 2'-O-methylation of cytidine and adenosine, but not guanosine. These results suggest that impairment in the 2'-O-methylation of nuclear RNA may be one of the major effects that limits the biological activity of rRNA and mRNA by these drugs.

5. The mechanism of action of XA and cycloleucine on the inhibition of methylation of nuclear RNA was assessed in L1210 cells in vitro. Both drugs

were found to inhibit base and 2'-O-methylation of nuclear RNA. XA inhibited RNA synthesis to an equal extent as base methylation but to a lesser extent than 2'-O-methylation. Cycloleucine inhibited base methylation more than 2'-O-methylation, but did not affect RNA synthesis. Cycloleucine inhibited the synthesis of S-adenosyl-L-methionine (SAM) in L1210 cells in a competitive manner, whereas XA exhibited a mixed-type of inhibition. Assay of the effects of these drugs on the activity of L-methionine:ATP adenosyltransferase in the 100,000 x g supernatant fluid of L1210 cells indicated that XA was not a direct inhibitor but that cycloleucine was a competitive inhibitor. These results indicate that the inhibitory action of cycloleucine and XA on the methylation of nuclear RNA is mediated via inhibition of SAM synthesis.

6. Cation exchange chromatography with SP-Sephadex was used to measure the levels of [^{3}H-methyl]S-adenosyl-L-methionine synthesized by L1210 cells *in vitro*. Separation of [^{3}H-methyl]S-adenosyl-L-methionine from [^{3}H-methyl] methionine and other metabolites was achieved by stepwise elution with varying concentrations of HCl.

PROPOSED COURSE:

Detailed analysis of RNA synthesis by the procedures outlined appears to be a sensitive and discriminating means of assessing the pharmacological action of nucleoside anticancer drugs. Further studies with agents such as 5-azacytidine and dihydro-5-azacytidine (synthesized in LMCB) will be undertaken to define their sites of action on the synthesis and processing of nRNA in L1210 cells.

Additional investigations of the synthesis of RNA *in vitro* will be carried out with L1210 cells metastasizing to the axillary lymph nodes in mice. This may serve as a sensitive means for assessing metastatic leukemic cells in various organ sites. The latter studies will be carried out in conjunction with survival studies using a metastatic L1210 tumor model system for measuring the activity of anticancer drugs. Modification of nucleoside anticancer agents to confer selective distribution of these drugs to metastatic sites will be carried out in conjunction with the Drug Design and Chemistry Section, LMCB.

PUBLICATIONS:

Glazer, R. I. and Peale, A. L.: Cordycepin and xylosyladenine: Inhibitors of methylation of nuclear RNA. *Biochem. Biophys. Res. Commun.* 81: 521-526, 1978.

Glazer, R. I.: The effects of 2'-deoxycoformycin on the action of cordycepin on nuclear RNA synthesis in regenerating liver. *Toxicol. Appl. Pharmacol.* (in press), 1978.

Glazer, R. I., Lott, T. J. and Peale, A. L.: Potentiation by 2'-deoxycoformycin of the inhibitory effect by cordycepin on nuclear RNA synthesis in L1210 cells *in vitro*. *Cancer Res.* (in press), 1978.

Peale, A. L. and Glazer, R. I.: Potentiation by 2'-deoxycoformycin of the inhibitory effect of xylosyladenine on nuclear RNA synthesis in L1210 cells *in vitro*. *Biochem. Pharmacol.* (in press), 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07110-02 NCI

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effect of Anticancer Drugs on the Translation of Messenger RNA *in vitro*

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Christine Carrico, Ph.D.	Pharmacology Research Associate	LMCB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Applied Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Antimetabolites such as 5-azacytidine and possibly also 5-fluorouracil are believed to exert antitumor effects, at least in part, through their incorporation into mRNA, and hence interference with the ability of mRNA to code for proteins critical to the balanced growth of the cell. This hypothesis will be tested by quantitative and qualitative assessment of the capacity of poly(A)mRNA from regenerating liver or L1210 cells to code for proteins in an *in vitro* heterologous wheat germ translation system. These studies will include an analysis of both the 5' cap and 3'-poly(A) structures of mRNA, as well as the synthesis of the mRNA *in vivo*. This experimental system will allow an assessment of the effect of anticancer drugs on the synthesis, post-transcriptional processing and coding properties of mRNA.

OBJECTIVES:

Many antimetabolites such as 5-fluorouracil (5-FU) and 5-azacytidine are believed to exert their antitumor effects, at least in part, by being incorporated into mRNA and initiating miscoding during translation. To test this hypothesis, poly(A)RNA from polyribosomes of regenerating rat liver or L1210 cells will be isolated and analyzed in an *in vitro* translation system prepared from wheat germ embryos. The labeled translation products will be analyzed by isoelectric focusing and polyacrylamide gel electrophoresis. In addition, rRNA and poly(A)RNA will be analyzed for chemical modification, as well as their rates of synthesis resulting from treatment with 5-FU and other anticancer drugs.

METHODS EMPLOYED:

Isolation of polyribosomes by Mg^{++} precipitation of 10,000 x g extracts of rat liver or L1210 cells; extraction of poly(A)RNA by SDS-phenol-HCCl$_3$ and poly(U)Sepharose chromatography; preparation of 23,000 x g extracts of wheat germ embryos; incubation of poly(A)RNA with [^{3}H]leucine or [^{35}S]methionine, amino acids, KCl GTP, ATP, PEP, Mg^{++}, spermidine and wheat germ extract; extraction of labeled proteins and analysis on slab or cylindrical polyacrylamide gels and analysis by fluorography and liquid scintillation counting; measurement of the 5'-cap by dihydroxyboronylphenylaminoethyl Sephadex chromatography and DEAE Sephadex-urea chromatography.

MAJOR FINDINGS:

1. Polyadenylic acid-containing RNA (poly(A)$^+$RNA) from regenerating liver of partially hepatectomized rats treated with 5-FU at 1 hr and at 16 hr after operation was translated in a cell-free wheat germ system. The products were analyzed by SDS-polyacrylamide gel electrophoresis, isoelectric focusing, and fluorography. Poly(A)$^+$RNA from 5-FU-treated rats (20 mg/kg) stimulated translation by 240 precent in comparison to poly(A)$^+$RNA from control animals. The stimulatory effect was associated with an overall increase in the quantity of products translated, as well as with a shift in the size of the proteins. No new translation products were observed. Interaction of the 5'-cap of the mRNA with the ribosome, as assessed by competition experiments with m^{7}GMP and M^{7}GDP, appeared to be unaltered in the 5-FU-modified poly(A)$^+$RNA.

2. The synthesis of polysomal RNA and the incorporation of [^{3}H]5-FU into RNA was measured. Non-poly(A)rRNA contained 0.45 ± 0.04 pmoles of 5-FU per µg RNA, while poly(A)RNA contained 2.68 ± 0.81 pmoles of 5-FU per µg RNA. Agarose-urea gel electrophoresis showed that poly(A)RNA labeled with [^{3}H]5-FU had a modal distribution in size of 21A and similar to that of control poly(A)RNA labeled with [^{3}H]orotic acid. rRNA labeled with [^{3}H]5-FU showed 45S, 28S and 18S RNA with an additional species at 38S in comparison to control rRNA. DEAE Sephadex-urea chromatography of alkaline hydrolysates of [^{3}H]5-FU-labeled rRNA indicated a greater distribution of label in the

dinucleotide fraction (20%)vs. that found in control rRNA (4%). Dihydroxyboronylphenylaminoethyl cellulose chromatography of [^{3}H]5-FU-labeled poly(A) indicated that 5-FU was present in internucleotide linkage and not in the 5'cap.

PROPOSED COURSE:

The effect of 5-azacytidine, as well as the synergistic regimen of thymidine plus 5-fluorouracil will be examined in a cell-free translation system with poly(A)-RNA from treated partially hepatectomized rats. These studies will be extended to tumor cells grown in suspension culture to correlate the mRNA-specific effects of these drugs with their growth inhibitory properties.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07111-02 LMCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Action of Anticancer Drugs on the Phosphorylation and Activity of Nonhistone Chromosomal Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Michel Legraverend, Ph.D.	Visiting Fellow	LMCB	NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Applied Pharmacology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The phosphorylation of nonhistone chromosomal proteins (NHCP) appears to exert a regulatory influence on transcription in eukaryotic cells. The recent discovery that cordycepin (3'-deoxyadenosine) can inhibit normal and neoplastic nuclear protein kinase activities in vitro has prompted an investigation of the role of phosphorylation in the action of this and related drugs. The phosphorylation of NHCP in vitro in isolated nuclei will be used to study the effect that cordycepin has on various classes of NHCP. These studies will be extended to L1210 cells in vitro to correlate the effects of cordycepin on nuclear phosphorylation and nRNA synthesis. The latter system will also allow a determination of the effect that the adenosine deaminase inhibitor, 2'-deoxycoformycin, has on the action mediated by cordycepin on the phosphorylation of NHCP. Further analysis of NHCP from rat liver will be performed in order to isolate those NHCP which stimulate transcription via RNA polymerase II in vitro. Characterization of stimulatory NHCP will be performed with specific antineoplastic drugs as pharmacological probes of their molecular sites of action in a cell-free transcription system.

-6040
(Rev. 10-76)

OBJECTIVES:

The phosphorylation of nonhistone chromosomal proteins (NHCP) is believed to exert a regulatory role on transcription in eukaryotic cells. This process is mediated via nuclear protein kinases, a heterogeneous group of isoenzymes which are cyclic nucleotide independent. Cordycepin (3'-deoxyadenosine) competitively inhibits protein kinase activities partially purified from rat liver or rat hepatoma 3024A. Two objectives of this project are: 1) to characterize the NHCP which are phosphorylated in vitro in isolated nuclei in the presence and absence of cordycepin, and 2) investigate the effect of cordycepin on the phosphorylation of NHCP in vitro in L1210 cells. The two aspects of this project will provide information about the mode of action of cordycepin in isolated nuclei and in intact cells on the phosphorylation of NHCP, and the relevance of this effect to its inhibitory action on RNA synthesis. A third objective is to isolate NHCP which specifically stimulate transcription in vitro. This will allow a detailed analysis of the components of a cell-free transcription system and the modulation of this system by anti-cancer agents.

METHODS EMPLOYED:

Isolation of nuclei from rat liver and L1210 cells by ultracentrifugation in hypertonic sucrose or by Triton X-100 extraction; labeling of NHCP in nuclei by incubation under optimal conditions with [γ-^{32}P]ATP or in intact cells by incubation with ^{32}P; extraction of NHCP with 7 M urea-phosphate buffer; characterization of NHCP by isoelectric focusing using 5% polyacrylamide gels with pH 3.5-10 and 4-6 ampholytes; purification of [^{32}P]-nuclear proteins by QAE-Sephadex chromatography prior to isoelectric focusing; preparative isoelectric focusing and two dimensional gel electrophoresis.

MAJOR FINDINGS:

1. The effects of cordycepin (3'-deoxyadenosine) and cordycepin triphosphate upon phosphorylation of nonhistone chromosomal proteins were assessed in isolated hepatic nuclei in vitro. Cordycepin and cordycepin triphosphate competitively inhibited phosphorylation of urea-soluble nuclear proteins with a K_i of 1.2×10^{-3} M and 8×10^{-5} M, respectively. Isoelectric focusing of urea-soluble proteins indicated that inhibition occurred predominantly in nuclear proteins with isoelectric points of pH 4-7. QAE-Sephadex chromatography of extracts of nuclei incubated with cordycepin and cordycepin triphosphate also showed inhibition of phosphorylation of nonhistone chromosomal proteins with similar isoelectric points, although greater resolution of proteins with isoelectric points of pH 6-7 was achieved. RNA polymerase I and II were not affected by cordycepin and cordycepin triphosphate after QAE-Sephadex chromatography of nuclear extracts incubated with either agent. However, RNA polymerase I and II in isolated nuclei were competitively inhibited by cordycepin triphosphate but not by cordycepin. These results suggest that cordycepin triphosphate, and perhaps cordycepin too, may affect transcription via interference with the phosphorylation of nonhistone chromosomal proteins.

2. Cordycepin and xylosyladenine were tested for their inhibitory effect upon the phosphorylation of nuclear proteins in L1210 cells in vitro. As indicated by isoelectric focusing of urea-soluble nuclear proteins with pH gradients of 3.5-10 and 4-6 phosphorylation of acidic nonhistone chromosomal proteins was strongly inhibited by both nucleosides, particularly in the presence of the adenosine deaminase inhibitor, 2'-deoxycoformycin. The inhibitory action of cordycepin and xylosyladenine was greater on NHCP of pI equal to 3-4.5 in comparison to NHCP of higher pI. The activity of the two inhibitors on these acidic species of NHCP correlated closely with their inhibitory action on RNA synthesis. These observations may explain, in part, the inhibitory effect of both drugs upon transcription.

PROPOSED COURSE:

NHCP which specifically stimulate transcription in vitro using heterogeneous RNA polymerase II and homologous chromatin will be characterized by ion exchange chromatography and preparative isoelectric focusing. Such a cell-free system will allow a detailed analysis of the interactions of its components with anticancer agents. Actinomycin D, α-amanitin, cordycepin 5'-triphosphate and O-n-octyloxime of 3'formylrifamycin SV (AF/013) will be used to assess interaction of NHCP with the DNA template, polymerase, nucleoside substrate site and initiation, respectively.

PUBLICATIONS:

Legraverend, M. and Glazer, R. I.: Inhibition of the phosphorylation of nonhistone chromosomal proteins of rat liver by cordycepin and cordycepin triphosphate. Cancer Res. 38: 1142-1146, 1978.

Legraverend, M. and Glazer, R. I.: Inhibition of the phosphorylation of nonhistone chromosomal proteins by cordycepin and xylosyladenine in L1210 cells in vitro. Mol. Pharmacol. (in press), 1978.

Drug Design and Chemistry Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1977 - September 30, 1978

Scope

The objectives of the Drug Design and Chemistry Section of the Laboratory of Medicinal Chemistry and Biology are: (1) chemical research in the design and synthesis of new drugs for the treatment of cancer, (2) the chemistry of agents and drugs of interest in the treatment of cancer, (3) research on the structure, purity, decomposition products and metabolites of antitumor drugs by analytical instrumental techniques, (4) the development of structure-activity techniques to summarize existing data and to provide guidelines for analog synthesis and (5) the promotion of the interdisciplinary approach to the synthesis and evaluation of rationally designed drugs by collaboration between chemical and biological scientists.

Staff

The staff consisted of four senior scientists, one scientist, one NIH Visiting Scientist, four NIH Visiting Fellows and one secretary.

Summary of Accomplishments

Approximately nine man-years of effort were devoted to intramural research projects described in Individual Project Reports Z01-CM-03580, Z01-CM-03581, and Z01-CM-03582 during the period covered. One agent developed somewhat earlier in these laboratories, AZQ (NSC 182986) was put into toxicology (DN2B) during this period. Spirohydantoin mustard (NSC 172112) remained in the DN2A category while attempts were made to resolve formulation difficulties. Dihydro-5-azacytidine (NSC 264880) continued in DN2A while biochemical comparisons with 5-azacytidine were investigated.

The synthesis, chemistry and biological activity of arabinosyl-5-azacytosine (ara-5AC) were studied. This compound, which combines half of the structure of ara-C with half of 5-azacytidine, has L1210 activity (QD 1-9 treatment schedule) which is as good or better than either parent in the same experiment. In addition, ara-5AC is active over an exceptionally wide dose range (200-0.78 mg/kg) on this schedule. Target specific agents for lung tumors and melanomas were studied. Transition state enzyme inhibitors were designed for use against cytidine deaminase, carbamylphosphate synthetase and aspartate transcarbamylase. Hydroxypyridones and catechol amines continued to provide P388 leukemia actives.

A partial elucidation of the mechanism of the mild oxidation reaction discovered and then used in the preparation of ara-5AC was carried out. Mass spectrometry was used extensively in the solution of structural and purity problems with both new and established antitumor agents.

Quantitative structure-activity correlations were started or completed for the platinum, colchicine, TEPA, adriamycin, aniline mustard, rifamycin, Cain acridine and podophyllotoxin families.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03580-09 LMCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Chemical Research in the Development of New Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. S. Driscoll	Head, Drug Design and Chem. Section	LMCB	NCI
OTHER:	J. A. Beisler	Research Chemist	LMCB	NCI
	R. W. Fuller	Chemist	LMCB	NCI
	J. A. Kelley	Research Chemist	LMCB	NCI
	M. M. Abbasi	Visiting Fellow	LMCB	NCI
	V. Feyns	Visiting Fellow	LMCB	NCI
	V. E. Marquez	Visiting Scientist	LMCB	NCI
	G. K. Menon	Visiting Fellow	LMCB	NCI
	M. Takai	Visiting Fellow	LMCB	NCI

COOPERATING UNIT (if any)

Drug Evaluation Branch, DTP, DCT, NCI
Lab. Chem. Pharmacology, DTP, DCT, NCI

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Drug Design and Chemistry

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 6.9	PROFESSIONAL: 5.9	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is the discovery of new types of clinically useful anticancer drugs. The following topics are of current interest: (1) nucleoside antimetabolites, especially 5-azacytidine analogues, (2) target specific agents for lung tumors and melanomas, (3) natural product analogues of usnic acid, chartreusin and colchicine, (4) transition-state enzyme inhibitors for cytidine deaminase and de novo pyrimidine biosynthesis enzymes and (5) pyridones and phenalkylamines as antitumor agents.

-6040
v. 10-

Project Description:

General Objective:

The objective of this project is the discovery of new types of clinically useful anticancer drugs. Medicinal chemical research is directed toward the synthesis of new compounds which have potential as useful agents. Leads for this program are generated from structure-activity studies, the DTP screening program and the literature.

Specific Objectives:

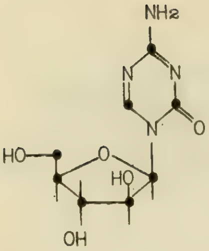
1. Synthesis and antitumor activity of triazine nucleosides and their reduced analogues;
2. Design and synthesis of target specific drugs, especially those directed to melanomas and tumors of the lung;
3. Preparation of analogues of natural products in order to overcome problems with the parent drugs;
4. Design and synthesis of transition-state enzyme inhibitors for pyrimidine deaminase and pyrimidine biosynthesis enzyme systems;
5. Exploitation of screening leads in the pyridone and phenalkylamine families.

Major Findings:

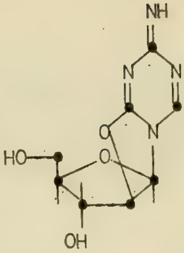
A. Nucleoside Antimetabolites

1. Arabinofuranosyl-5-azacytosine (Drs. Abbasi, Beisler): The successful synthesis in our laboratory of arabinofuranosyl-5-azacytosine (A-1) made possible the collection of substantial amounts of biological data relevant to antitumor activity. In a direct comparison study with 5-azacytidine (5-AC) using the L1210 system, QD 1-9, A-1 gave 147, 144 and 170 %ILS while 5-AC gave 134, 126 and 194 %ILS in respective controls. In comparison to an optimal dose of 3 mg/kg for 5-AC, the optimal dose of A-1 was about 50 mg/kg. In good agreement with *in vivo* testing, the cytotoxicity of 5-AC against cultured L1210 cells was found to be about a log unit more potent than A-1. An L1210 subline resistant to 5-AC was also not sensitive to A-1 in an *in vivo* assay. Similarly, cross-resistance of A-1 with cytosine arabinoside was also demonstrated *in vivo* with an appropriate L1210 subline. While the 5,6-dihydro derivative of 5-AC has substantial activity in the L1210 system, the analogous dihydro derivative of A-1 was devoid of activity.

2. Anhydro-5-azacytidine (Drs. Abbasi, Beisler): As part of a continuing interest in analogs of 5-azacytidine, a synthesis of 2,2'-anhydro-1-(β-D-arabinofuranosyl)-5-azacytosine (A-2) was initiated. A-2 is the 5-aza analogue of cyclocytidine. Accordingly, 5,6-dihydro-5-azacytidine was treated with 2-acetoxyisobutyryl chloride to give A-3 after hydrolysis of the substituents from the 5'- and 3'-positions. No activity was shown by this dihydro precursor of A-2 against the L1210 system (200-1.56 mg/kg).



A-1



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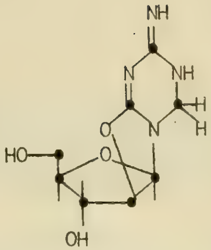
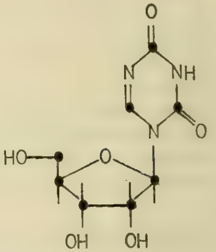
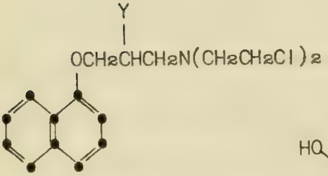


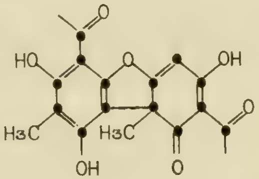
A-3



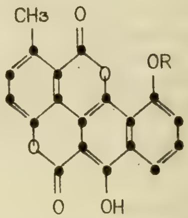
A-4



B-1, Y = OH
B-2, Y = Cl

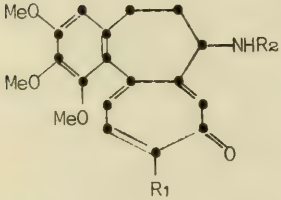


B-3

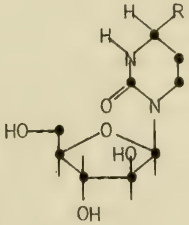


R = D-fucosyl-D-digitalosyl

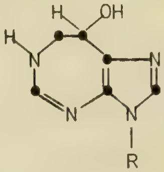
C-1



C-2

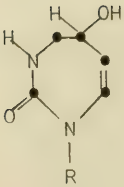


D-1, R = OH
D-2, R = $NHCOCH_3$

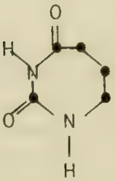


R = 2'-deoxyribose

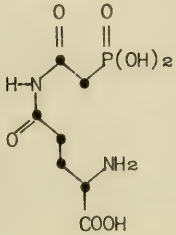
D-3



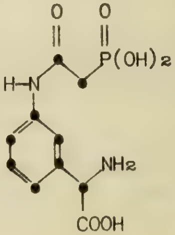
D-4



D-5



D-6



D-7

This is consistent with the lack of activity found for the dihydro derivative of arabinofuranosyl-5-azacytosine. Completion of the synthesis requires dehydrogenation of the triazine nucleus. To date no oxidizing agent has been found which would enable the desired chemical transformation. The trimethylsilylation method, which was successfully used for the synthesis of arabinofuranosyl-5-azacytosine, failed to provide A-2. This project will be terminated if a suitable oxidizing agent is not soon found.

3. 5-Azauridine (Drs. Abbasi, Beisler): It is not known whether enzymatic deamination of 5-AC to form 5-azauridine (A-4) is an inactivation process or if A-4 contributes to the antitumor activity of 5-AC. In order to explore that question, a short synthesis of A-4 was conceived so that sufficient amounts of the nucleoside could be entered into the program for *in vivo* studies. Unfortunately, synthetic attempts to prepare this very unstable molecule failed. Synthetic work in this area has been terminated.

B. Target Specific Agents

1. Lung Antitumor Agents

a. Propranolol Mustard (Drs. Feyns, Beisler): Since propranolol is known to concentrate in lung tissue, Dr. Adamson (LCP) suggested a mustard analog of propranolol (B-1) as a potential lung tumor agent. A selective chlorination of a triol intermediate using tosyl chloride in DMF solution gave the target compound (B-1) in moderate yield. This compound gave 46% ILS in the P388 test system. Non-selective chlorination resulted with thionyl chloride to give B-2. This trichloro compound exhibited similar activity against P388 leukemia (41%). Both the dichloro compound, B-1, and the trichloro compound, B-2, have been scheduled for testing against Lewis lung carcinoma.

b. Usnic Acid Derivatives (Drs. Takai, Beisler): A lichen metabolite, usnic acid (B-3), showed low level activity in both Lewis lung and the P388 leukemia test systems. Since usnic acid is commercially available, a program was initiated to synthesize derivatives of the natural product with the goal of developing a potential lung tumor agent. Simple derivatives of B-3 had no Lewis lung activity, but in some cases, weak P388 activity. Since the eight synthetic materials had log P values not appreciably different from that of usnic acid (log P 2.88), two additional glycoside derivatives with more hydrophylic character were synthesized in a six step procedure. The glycosides of B-3 gave measured log P values of ca. 0.5. Unlike B-3, which is very water insoluble, the glycoside derivatives showed a good solubility in water. Nevertheless the glycoside derivatives showed no activity against P388 leukemia. No additional work is planned in this project area.

2. Antimelanoma Agents (Dr. Driscoll)

a. Phenalkylamines: Based on the role of catechol amines and their precursors in melanin formation and our discovery that dopamine as well as several other phenalkylamines possess L1210 and P388 leukemia activity, approximately 40 phenalkylamines are being evaluated against P388 leukemia and B16 melanoma in a structure-activity study.

b. Studies based on melanoma tyrosinase activity: It has been reported that melanoma cells concentrate DOPA and its precursors. In cooperation with Dr. Mary Wolpert (LMCB), a report that L-DOPA methyl ester is effective against murine tumors is being checked. In an attempt to exploit the tyrosinase activity in melanomas, tyrosine nitrogen mustard is being tested against B16 melanoma. Several *in vivo* P388 active catechol amines are being studied *in vitro* by Dr. Breitman (LMCB) in both melanotic and amelanotic cell lines.

C. Chemical Modification of Antitumor Natural Products

1. Chartreusin (Drs. Takai, Beisler): Chartreusin (C-1) is a fermentation product that achieved DN2A status in the program on the basis of its L1210, P388 and B16 activity. Subsequently, it was found to be rapidly excreted in bile and also difficult to formulate because of poor water solubility. A synthesis project was initiated for the purpose of replacing the unusual disaccharide moiety of C-1 with the readily available disaccharide, D-maltose. The resulting derivative might be expected to have a greated water solubility because of the increased number of hydroxyl groups in the molecule. The structure of the proposed synthetic derivative, however, has many similarities with the parent (including the α-disaccharide linkage). Unless the unusual disaccharide portion of C-1 plays a crucial role in the antitumor activity of C-1, the synthetic derivative might retain the desirable biological properties of the parent while lacking its undesirable bilary excretion properties.

2. Colchicine (Drs. Beisler, Quinn): Regression analysis of 15 colchicine derivatives gave a good correlation of biological activity with partition coefficient (log P) and with substitution on the nitrogen atom. While maintaining proposed derivatives within the ideal log P range of 1.5 to 1.9, various N-substituted analogues are being synthesized, e.g. C-2 (R_1 = OMe, R_2 = $COCH_2CN$) and (R_1 = OMe, R_2 = $COCF_3$). Although not suggested by the regression equation, the effect of electronic effects on the tropolone ring will be probed through derivatives such as C-2 (R_1 = SO_2Me, R_2 = $COCH_3$) and (R_1 = SOMe, R_2 = $COCH_3$).

D. Transition-State Enzyme Inhibitors

1. Cytidine Deaminase Inhibitors Based on Reduced Ara-C and Ara-U (Drs. Menon, Kelley): The synthesis of the arabinosyl analogue (D-1) of tetrahydrouridine (THU) has been undertaken in an attempt to develop a more effective cytidine deaminase inhibitor. Several reduced derivatives of both Ara-C and Ara-U and one monoacetylated tetrahydro derivative of ara-C (D-2) have been prepared in gram quantities; testing is underway. Deblocking and hydrolyzing D-2 to produce the targets ara-tetrahydrocytosine and ara-THU have proven difficult. Present synthetic work is directed toward achieving

these transformations as well as following an alternate reductive approach to produce the desired compound from our recently synthesized triacetate of dihydro-ara-U (NSC 299556).

Methoxylamine hydrochloride reacts selectively with the C-4 amino functionality of cytidine to form the corresponding methoxime, even in the presence of other DNA nucleosides. Since the trimethylsilyl derivative of this methoxime nucleoside offers enhanced chromatographic and mass spectral properties, it was decided to study the utility of this reagent in regard to other cytidine nucleosides as well as the antitumor properties, if any, of these compounds. The methoximes of cytidine and ara-C have been synthesized in good yield and are undergoing *in vivo* testing. Present work is concerned with evaluating the suitability of this reagent for derivatizing a wide range of cytidine nucleosides on an analytical scale.

2. Cytidine Deaminase Inhibitors Based on 2'-Deoxycoformycin (Dr. Marquez): The purine deaminase inhibitor, 2'-deoxycoformycin (D-3), with a K_i value of 2×10^{-12} M, is much more potent than the best pyrimidine deaminase inhibitor, THU (K_i 10^{-8} M). The unusual structural feature of D-3 is its expanded seven member ring in the purine base. If this feature should be responsible for the remarkable K_i value for D-3, a reasonable approach to an inhibitor of cytidine deaminase would involve the synthesis of the corresponding seven-member pyrimidine nucleoside (D-4).

Multiple synthetic approaches have been utilized in attempts to prepare the seven-member pyrimidine ring of D-4. Several unexpected rearrangement reactions have been discovered during these studies. The most promising approach at the present time utilizes a synthesis of squamolone (D-5). Current efforts are directed towards the preparation of the furanosyl nucleosides of D-5 which will be converted to D-4 and its analogues.

3. Inhibitors of the Enzymes Involved in De Novo Pyrimidine Biosynthesis (Drs. Marquez and Feyns): The enzymes which catalyze the first two steps in pyrimidine biosynthesis are carbamyl phosphate synthetase (CPase) and aspartate transcarbamylase. Since a compound is known (PALA) which is an efficient inhibitor of the latter enzyme, we have undertaken the preparation of inhibitors for CPase, the first enzyme in the sequence. The compound best approximating the postulated transition-state intermediate in the biosynthesis of carbamyl phosphate is PALG (D-6). This compound is much more difficult to synthesize than PALA since the phosphonoacetyl group must be added to an amide nitrogen rather than an amino nitrogen. An analogue (D-7) of PALG has been synthesized and is scheduled for CPase inhibition studies in collaboration with Dr. Cooney (LT). The *para* isomer of D-7 was also prepared and will be evaluated to determine the effect of structure on activity. A third compound in the series was prepared and evaluated. It inhibited CPase (78% at 10^{-2} M) while having little effect (9%) on aspartate transcarbamylase at the same concentration. The preparation of PALG (D-6) continues to be our ultimate synthetic objective on this project.

E. Development of Screening Leads

1. Hydroxypyridones (Drs. Hwang, Driscoll): Alpha-hydroxy-4-pyridone derivatives were prepared and found to be active against P388 leukemia (ILS 80%). Synthesis and structure-activity correlations are continuing in this series.

Publications:

Driscoll, J.S. and Beisler, J.A.: Antineoplastic Agents. In Clarke, F.H. (Ed.): Annual Reports in Medicinal Chemistry, Vol. 12, Academic Press, N.Y., 1977, p. 120-129.

Beisler, J.A., Peng, G.W. and Driscoll, J.S.: Potential Antitumor Agents. Procarbazine Analogs and other Methylhydrazine Derivatives. J. Pharmaceutical Sci., 66: 849-852, 1977.

Szekerke, M. and Driscoll, J.S.: The Use of Macromolecules as Carriers of Antitumor Drugs. European J. Cancer, 13: 529-537, 1977.

Beisler, J.A., Abbasi, M.M., Kelley, J.A. and Driscoll, J.S.: Synthesis and Antitumor Activity of Dihydro-5-azacytidine. A Hydrolytically Stable Analogue of 5-Azacytidine. J. Medicinal Chem., 20: 806-812, 1977.

Beisler, J.A., Abbasi, M.M. and Driscoll, J.S.: The Synthesis and Antitumor Activity of Arabinosyl-5-azacytosine. Biochem. Pharmacol., 26: 2469-2472, 1977.

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Beisler, J.A. Abbasi, M.M. and Driscoll, J.S.: Synthesis, Structure and Antitumor Activity of 5,6-Dihydro-5-azacytidine. U. S. Patent 4,058,602, November 15, 1977.

Driscoll, J.S., Melnick, N.R., Quinn, F.R., Lomax, N., Davignon, J.P., Ing, R., Abbott, B.J., Congleton, G. and Dudeck, L.: Psychotropic Drugs as Potential Antitumor Agents. A Selective Screening Study. Cancer Treat. Rept, 62: 45-74, 1977.

Hirata, T., Peng, G. and Driscoll, J.S.: Potential Central Nervous System Antitumor Agents V. Phenothiazines II. Fluphenazine Analogues. J. Pharmaceutical Sci., 67: 157-162, 1978.

Futterman, B., Derr, C.J., Beisler, J.A., Abbasi, M.M. and Voytek, P.: Studies on the Cytostatic Action, Phosphorylation and Deamination of 5-Azacytidine and 5,6-Dihydro-5-azacytidine in HeLa Cells. Biochem. Pharmacol., in press.

Hwang, D.R. and Driscoll, J.S.: Pyridones as Potential Antitumor Agents. J. Medicinal Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03581-09 LMCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Analytical Chemistry of New Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Kelley	Research Chemist	LMCB	NCI
OTHER:	J. S. Driscoll	Head, Drug Design and Chem. Section	LMCB	NCI
	G. K. Menon	Visiting Fellow	LMCB	NCI

COOPERATING UNITS (if any)

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Pharmaceutical Resources Branch, DTP, DCT, NCI

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Drug Design and Chemistry

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is the research and development of analytical techniques which are used to: (1) establish the structure and purity of new antitumor agents and their metabolites, (2) determine physical and chemical properties of new anticancer drugs and (3) study reaction mechanisms. Mass spectrometry, gas-liquid chromatography and the combination of these techniques is emphasized. Compounds of current interest are 5-azacytidine, reduced nucleosides, hydantoins, and aziridinylquinones. Silation mediated oxidation reactions are under investigation.

PHS-6040
(Rev. 10-76)

Project Description:

General Objectives:

The objective of this project is the research and development of analytical techniques which are used to:

1. establish the structure and purity of new anticancer drug candidates;
2. establish structures of metabolites of new antitumor agents;
3. determine important physical and chemical properties of new anticancer drugs; and
4. study reaction mechanisms.

Methods Employed:

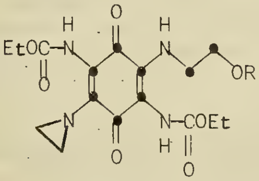
Mass spectrometry (MS), gas-liquid chromatography (GC) and the combination of these techniques (GC/MS) are emphasized. Other analytical methods such as high pressure liquid chromatography (HPLC), NMR, UV and IR spectroscopy also are employed.

Major Findings:

I. Research Problems

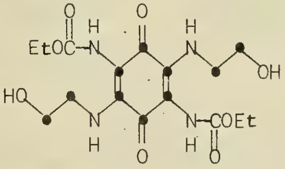
A. Silylation-Mediated Oxidation (Drs. Kelley and Abbasi): Trimethylsilylation is widely employed to derivatize non-volatile compounds of biomedical interest (*e.g.* amino acids, nucleosides, sugars) for analysis by gas chromatography (GC) and combined gas chromatography-mass spectrometry (GC-MS). A novel oxidation that quantitatively dehydrogenated either 5,6-dihydropyrimidine and *s*-triazine bases or their respective nucleosides under mild conditions was discovered during the course of such an analytical trimethylsilylation. The kinetics, mechanism, scope and synthetic utility of this new reaction were investigated for reduced nucleoside substrates by quantitative gas chromatographic analyses employing internal standards. For conversion of dihydro-5-azacytidine to 5-azacytidine (5-AC), the oxidation followed pseudo first order kinetics with a half-life of 2.02 hr. at 76.5° ± 1° and required molecular oxygen as well as reagents capable of both N- and O-trimethylsilylation. Trace heavy metal impurities (Cu,Fe) which might catalyze the oxidation were not present, but the reaction was accelerated by heat or the addition of free radical initiators. Classical chemical dehydrogenating agents such as DDQ, chloranil and MnO_2 were unable to effect this same oxidation even under vigorous conditions. The rate of reaction was also dependent on the chemical identity of the base and sugar moieties of the dihydronucleosides. For nucleosides containing 5,6-dihydro-5-azacytosine, the oxidation of the arabinofuranosyl analogue was approximately four times slower than that of the corresponding ribofuranosyl compound. Dihydropyrimidines (*e.g.* dihydrouridine) were also dehydrogenated at substantially slower rates than dihydro-*s*-triazines. The utility of this unique oxidation was demonstrated in the synthesis of the new nucleoside arabinofuranosyl-5-azacytosine where GC-MS studies were used to optimize reaction conditions and optimize products. Application of this unique reaction to O^2,2'-anhydro-5,6-dihydro-5-azacytidine has so far failed to yield the desired anhydro-5-azacytidine. Instead, combined oxidation and rearrangement produces a fluorinated derivative of the target compound which has incorporated the elements of CF_3CN from the silylating/oxidizing reagent. Studies are underway to completely elucidate the structure of this material as well as to obtain the desired anhydro-nucleoside. When either ribofuranosyl or arabinofuranosyl dihydro-5-azacytidine-6-d_1 was oxidized, deuterium was preferentially retained at the 6-position of the base with an isotope effect of 2.8 ± 0.3. Thus the feasibility of synthesizing nonexchangeable deuterium or tritium labelled drugs from their reduced precursors in the appropriate chemical systems is being investigated.

B. AZQ (NSC 182986) Contaminants and Decomposition Products (Drs. Kelley and Poochikian, PRB): The major contaminant (approximately 5% by weight) in synthetic AZQ was identified as 2,5-diamino-3,6-dichloro-1,4-benzoquinone. High pressure liquid chromatography was employed to isolate microgram amounts of the aqueous acidic degradation products of AZQ that would result from different modes of formulation. Mass spectrometry, coupled with suitable chemical derivatization for volatility and functional group specificity, was employed for identification of these isolated substances after an initial mass spectral study of model compounds. The primary decomposition product was the amino alcohol 1 resulting from hydrolysis of a single aziridine ring, while the completely ring-opened amino alcohol 2 was the final product. Trace amounts of the amino ethyl ether 3, which could arise as a by-product during the recrystallization of AZQ from ethanol, and di-(2-ethylhexyl)-phthalate, an ubiquitous plasticizer, were also detected.



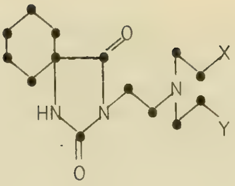
1: R = H

3: R = Et



2

C. Spirohydantoin Mustard Hydrolysis (Drs. Kelley and Flora, PRB): A quantitative gas chromatographic assay using an internal standard was developed for the direct analysis of spirohydantoin mustard (4) (SHM_2, NSC 172112) and its primary aqueous hydrolysis products (5,6). The kinetics of SHM_2 hydrolysis were studied over the range pH 4-6 in aqueous 10% dimethylacetamide at 25° to duplicate formulation conditions. Mustard half-lives



4: X,Y = Cl

5: X = Cl, Y = OH

6: X,Y = OH

were substantially less than those inferred from titration of free chloride ion; a half-life of 13.8 min. was obseaved at pH 6 while buffering the solution to pH 4 only increased it to 17.3 min. Good agreement for free chloride ion concentration was obtained between values calculated from GC data or determined directly.

D. Pyridyloxadiazoles (Drs. Kelley and Marquez): The characterization of a series of isomeric pyridyloxadiazoles and their unexpected rearrangement products was accomplished.

II. Synthetic Project Support (Dr. Kelley)

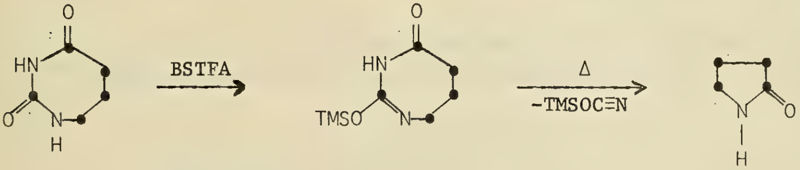
A. Phosphorus Containing Pyrimidine Biosynthesis Inhibitors: The intermediates and products in the synthesis of phosphonoacetyl derivatives of aminophthalic acid and aminophenyl glycine were characterized by mass spectrometry and combined GC-MS where appropriate to determine purity and confirm structure.

B. Propranolol Mustard: High resolution mass spectrometry coupled with computer-assisted analysis was employed to characterize in detail the mass spectral fragmentation behavior of propranolol mustard and the corresponding trichloride.

C. Usnic Acid: Glycosides of usnic acid derivatives were examined after pertrimethylsilylation. A series of acetylated usnic acid oxazole derivatives were analyzed to corroborate the proposed structures.

D. NSC 50741: Headspace analysis of the volatile decomposition products of this one arm sugar mustard after prolonged room temperature storage revealed acetone and a trace of methanol.

E. Pyrimidine Deaminase Inhibitors: Synthetic intermediates of the aglycones of the target nucleosides were characterized by mass spectrometry and combined GC-MS. A product thought to be a cyclic urea or the isomeric cyclic carbamyl amine was shown instead to be a rearranged aminolactone. The structure of squamolone (7), the 7-membered ring analog of dihydrouracil, was confirmed by mass spectrometry. Analysis of trimethylsilylated squamolone (8) revealed a thermally-induced elimination of trimethylsilyl hydrocyanic acid to give the ring-contracted 2-pyrrolidinone. The scope and applicability of this reaction is being explored.



F. 1,4-Benzoquinones: The mass spectrum of 2,5-dichloro-3,6-diisocyano-1,4-quinone, a compound with no proton NMR spectrum, was analyzed in detail to substantiate the structure.

G. Colchicine: Mass spectral analysis of a colchicine derivative showed it to be a sulfone rather than a sulfoxide.

Publications:

Marquez, V.E., DiParsia, M.T. and Kelley, J.A.: Synthesis of Pyridyloxadiazoles 1. Characterization and Thermal Rearrangement of an Unexpected 1,2,4-Oxadiazol-5(4H)one. J. Heterocyclic Chem. 14: 1427-1429, 1977.

Okada, K., Kelley, J.A. and Driscoll, J.S.: Intramolecular Cyclizations Leading to Bridgehead Bicyclics 2. 5,5-Dialkylhydantoin Derivatives. J. Heterocyclic Chem. 14: 511-513, 1977.

Okada, K., Kelley, J.A. and Driscoll, J.S.: Intramolecular Cyclizations Leading to Bridgehead Bicyclics 3. 5,5-Diphenyl-2-thiohydantoin Derivatives. J. Organic Chem. 42: 2594-2597, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03582-04 LMCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Structure-Activity Studies of Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. R. Quinn	Chemist	LMCB	NCI
OTHER:	J. S. Driscoll	Head, Drug Design and Chem. Section	LMCB	NCI
	J. A. Beisler	Chemist	LMCB	NCI

COOPERATING UNITS (if any)

Pomona College

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Drug Design and Chemistry

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.1	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are: (1) the development of quantitative structure-activity correlations among antitumor agent congeners, (2) the development of general structure-activity relationships among large numbers of unrelated antitumor agents and (3) the use of the correlation data obtained to generate leads for new synthesis and increase the rationale for the testing of new agents. Compound families of current interest are cis-platinum, colchicine, TEPA, adriamycin, aniline mustard, rifamycin, Cain acridine and podophyllotoxin.

Project Description:

General Objectives:

The objectives of this project are:

1. the development of quantitative correlations of antitumor and toxicity data with structural features among antitumor agent congeners;
2. the investigation of structure-activity techniques as applied to antitumor data;
3. the development of correlations between structure and antitumor activity among large numbers of structurally unrelated compounds;
4. the use of the correlations obtained to: (a) generate leads for new synthesis, (b) increase the rationale for selection of new compounds to be tested and (c) reduce the number of synthetic analogs necessary to obtain the optimally active member of a new series.

Methods Employed:

Computer techniques are used to analyze and organize biological screening data. These data are then correlated with molecular structural parameters using computerized regression analysis techniques.

Major Findings:

I. Quantitative Structure-Activity Relationships by the Hansch Technique

A. Intramural Studies (Dr. Quinn)

1. Rifamycin analogs: A cooperative project with Dr. M. Wolpert (LMCB) is being completed. Inhibition of SSV-DNA α-polymerase, β-polymerase, and reverse transcriptase were measured and a correlation has been obtained in terms of log P and $(\log P)^2$. This result quantitatively confirms the findings of Calvin, Gallo and other investigators. Indications are that the compounds predicted as optimal may be too lipophilic ($\log P > 6$) to be clinically useful.

2. Colchicine analogs (Dr. Beisler): A study was initiated to correlate the effect of structural variations on the *in vivo* P388 activity of this series. A good correlation ($r = 0.952$, $s = 0.356$) has been obtained showing a dependence on log P, $(\log P)^2$ and the beneficial effect of an electron-withdrawing substituent on the nitrogen atom. Inclusion of data for the compound given a clinical trial, trimethyl colchicinic acid methyl ether (NSC 36354), indicates that it is not the best analog, either in terms of log P or structure. A number of key analogs are being synthesized to confirm the correlation and to probe for possible hidden steric or electronic effects.

3. Adriamycin analogs: A study is in progress to investigate the effect of structural variation on the solid tumor (B16 melanoma) activity of this

series. An attempt to relate Prof. Zbinden's cardiotoxicity data with structural parameters has yielded a preliminary, but statistically significant, equation. Indications are that toxicity is decreased with increased log P. The replacement of oxygen by an imino (=NH) group in the 11-position also appears to be favorable.

4. Podophyllotoxin analogs: This series of analogs, which includes VM-26, is being investigated in an attempt to correlate structural parameters with P388 activity.

B. Contract Studies (Drs. Hansch, Quinn)

1. Aniline mustards: A study was recently published in which correlations of these congeners in Walker 256, L1210 and P388 were presented. Further work is in progress to determine the optimum log P of alkylating agents against solid tumors. Twenty compounds were synthesized and their substituent constants were measured. These compounds have been submitted for testing against the L1210, P388, B16 and Colon 38 tumor models. These twenty compounds also have been assayed for mutagenicity by means of the Ames Test. Indications are that mutagenicity of these alkylating agents decreases with an increase in log P and with the presence of electron-withdrawing groups. These same compounds are being tested for carcinogenicity in the pulmonary adenoma assay in mice.

2. Phenyl triazenes: A study of the antitumor activity of phenyl triazenes was completed and accepted for publication. The conclusion is that for phenyl, pyrazolyl and imidazole triazenes the ideal lipophilicity (log P_0) is approximately 1. Electron-releasing substituents increase potency. A set of phenyl triazenes has been tested for mutagenicity in the Ames Test. A correlation has been obtained and, as in the case of the aniline mustards, mutagenicity decreases with an increase in log P and with the presence of electron-withdrawing groups.

3. TEPA analogs: A significant ($r = 0.93$, $s = 0.24$) correlation has been obtained for 39 TEPA analogs against P388 leukemia *in vivo*. The activity of these compounds is a function of $(\log P)^2$ and decreases with the presence of electron-releasing groups on the aziridine rings. The ideal log P, as for other alkylating agents against leukemias, is low ($\log P_0 = 0$). Work is continuing to accurately determine the partition coefficients of these materials.

4. cis-Platinum analogs: A study has been initiated to quantitatively determine the structural parameters which govern the activity of these materials. A number of compounds have been synthesized for determination of substituent constants and for screening in the P388, L1210 and B16 tumor models.

5. Adriamycin analogs: Work is being completed on a study of the activity of these anthracyclines in both early and advanced P388. A number of partition coefficients have been measured in an attempt to verify the minimal dependence on log P.

6. Cain Acridines: A study is in progress to discover a structure-activity relationship for this series and to determine whether the optimum compound has been made.

7. Terephthalanilides: A study of the L1210 activity of these materials is being prepared for publication. This study resulted in the recommendation of one compound (NSC 125030) for the tumor panel. This compound was active in five of the six panel test systems.

Publications:

Panthananickal, A., Hansch, C., Leo, A. and Quinn, F.R.: Structure-activity relationships in antitumor aniline mustards. J. Medicinal Chem. 21: 16-26, 1978.

Hatheway, G., Hansch, C., Kim, K., Milstein, S., Schmidt, C., Smith, R. and Quinn, F.: Antitumor 1-(X-aryl)-3,3-dialkyltriazenes. I. Quantitative structure-activity relationships vs L1210 leukemia in mice. J. Medicinal Chem., in press.

Hansch, C., Hatheway, G., Quinn, F. and Greenberg, N.: Antitumor 1-(X-aryl-3,3-dialkyltriazenes. II. On the role of correlation analysis in decision making in drug modification. Toxicity QSAR of 1(X-phenyl-3,3-dialkyltriazenes in mice. J. Medicinal Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03579-05 LMCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effect of Chemotherapeutic Agents on L1210 Cell Cycle Progression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Vincent H. Bono, Jr.	Chief	IDB NCI
OTHER:	Robert L. Dion	Chemist	LMCB NCI

COOPERATING UNITS (if any)

The Upjohn Company, Kalamazoo, Michigan, Cancer Research Contract N01-CM-43743

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 1/4	1 1/4	

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project is designed to investigate the effects of cancer chemotherapeutic agents on cell cycle progression based on their cytotoxicity in an in vitro L1210 murine leukemia system. DNA content distribution (DCD) curves are generated with the use of a flow microfluorometer and drug treated curves are compared with steady state DCD curves after analysis by computer. An attempt to classify these agents as to their mode of action (anti-metabolite, etc.) by computer analysis is in progress.

Project Description:

This project is concerned with development of techniques for the analysis of drug effects on the progress of cells through the proliferative cell cycle and their application to (a) the characterization of therapeutically active drugs and (b) the identification and possible action of agents with potential therapeutic activity. The major methods employed are (a) mammalian cell culture (L1210 murine leukemia in suspension), (b) flow microfluorometric determination of cell DNA content using hypotonic citrate/propidium iodide stained cells in a Cytofluorograf (Biophysics System; Model 4801) interfaced with a multichannel pulse height analyzer and (c) processing, analyzing, and plotting cell DNA content distributions (DCD) by computer.

During the current year, 12 non-parenteral lots of methotrexate (NSC-740) containing various amounts and types of contaminants were assayed for the reversibility of toxic effects by the presence of leucovorin (Ca^{++}). Concentrations used were those which would emulate current "High Dose MTX" clinical protocols. Single lots of the 3',5'-dichloro analog (NSC-29630) and tetrahydrohomofolate (NSC-89473) were also assayed with this system.

Eighteen additional drugs were evaluated for their effects on L1210 cell cycle progression: dibromomannitol (NSC-94100), dibromogalactitol (NSC-104800), prospidine (NSC-166100), dianhydrogalactitol (NSC-132313), indicine-N-oxide (NSC-132319), gallium nitrate (NSC-15200), hexamethylmelamine (NSC-13875), neocarzinostatin (NSC-157365), anguidine (NSC-141537), bruceantin (NSC-165563), pyrazolo imidazole (NSC-51143), PCNU (NSC-95466), dihydro-5-azacytidine (NSC-264800), nogalamycin (NSC-70845), 7-O-CH_3-nogarol (NSC-293863), 7-O-CH_3-nogalarol (NSC-293862) and nogamycin (NSC-268450).

Preliminary studies were made to assess the feasibility of evaluating chemotherapeutic effects of antineoplastic agents on solid tumors in mice using DNA content distribution (DCD). The tumors investigated were (a) B16 melanoma, (b) colon 26, and (c) colon 38. This necessitated evaluation of a homogenization/detergent lysis technique rather than the hypotonic lysis technique used with cell culture in order to potentiate enzyme denaturation and release of parenchymal cells. In addition to tumors, normal organ tissues such as spleen, heart, liver, and thymus were examined. Concomitantly with the latter studies, several attempts were made at doing sequential biopsies on individual, anesthetized mice with AMSA (NSC-249992) treated B16 melanoma. The ultimate goal of these studies was to develop the techniques to apply to clinical therapy studies of head and neck solid tumors.

We acquired a Tektronix 4051 minicomputer which was interfaced with X-Y plotter and pulse height analyzer and have developed the necessary software for the real time acquisition and magnetic tape storage of DCD data. The software for processing and plotting the DCD profiles was also written, resulting in virtual complete independence of the DEC-10 main frame computer. Software for doing linear regression analysis was also developed.

5

Major Findings:

The toxic effects of Methotrexate (85-90% purity) up to 1.3 x 10^{-4}M could be ~90% reversed by 1.5 x 10^{-3}M leucovorin; at 1.1 x 10^{-5}M MTX in the presence of 1.5 x 10^{-3}M leucovorin (10% growth rate inhibition of L1210) there were striking kinetic changes in DCD curves. There appeared to be little or no non-CF reversing material. This study was reported at the American Association for Cancer Research Meetings, April 5-8, 1978 in Washington, D.C. Dichloromethotrexate had 3 times the potency of MTX on a molar basis in the presence of leucovorin. Tetrahydrohomofolate was not reversed by leucovorin or L-methionine at the ID_{50} dosage.

NSC	Name	L1210 ID_{50} (μg/ml)	Primary DCD Effects
94100	Dibromomannitol	152	Late S; G2 block at 24 hrs.
104800	Dibromogalactitol	43	"
166100	Prospidine	605	N.A.*
132313	Dianhydrogalactitol	2.4	Late S; G2 block at 24 hrs.
132319	Indicine-N-oxide	160	N.A.*
15200	Gallium Nitrate	120	N.A.*†
13875	Hexamethylmelamine	80	N.A.*
157365	Neocarzinostatin	0.04	G2 arrent
141537	Anguidine	0.0095	G1/S block
165563	Bruceantin	0.014	" "
51143	Pyrazolo-imidazole	115	Slow traverse thru S
95446	PCNU	3.8	Slow traverse thru S; G2 block

*N.A. DCD not run because of low potencies
†S.R.I reports an ID_{50} of 16 μg/ml

Nogalamycin and its three analogs were analyzed as part of a collaborative study with Dr. Bijoy Bhuyan of the Upjohn Company. L1210 cells were exposed to ID_{25}, ID_{50}, and ID_{90} dosages of the four compounds and aliquots for analysis for 2, 8, and 24 hours. Effects on cell cycle distribution were time/dose dependent. Nogalamycin (NSC-70845 showed no striking changes over 24 hour period. 7-O-CH_3-Nogarol (7-O-MEN) appeared to retard transit through G2. 7-O-CH_3-Nogalorol appears to inhibit transit through S-phase with degradation of DNA at the high dose after 24 hours. Nogamycin showed only slight changes at the lowest dose. The ID_{25} and ID_{50} dosages at 24 hours showed the beginning of and extensive DNA degradation respectively.

Mouse liver (BDF/control) DCD curves were bimodal with a channel ratio of 2 and peak height ratios (G1/G2) varying from ~2 to 4. Heart, lung, thymus, kidney, and spleen gave primarily unimodal peaks. Long term effects of AMSA on B16 melanoma (primarily unimodal DCD) were unsuccessful because dosage data were derived from saline suspension of drug injected rather than a 50% DMSO/H_2O solution. Presumably, this resulted in a higher absorption rate with much greater toxicity Injected mice died on days 1 to 4.

Untreated colon 26 gives a bimodal DCD curve with G1 and G2 peak height ratios carrying from 1 to 4 with very few cells in S-phase.

Publications:

Fernandez-Pol, J.A., Bono, V.H., Jr., and Johnson, G.S.: Control of growth by picolinic acid: Differential response of normal and transformed cells. Proc. Natl. Acad. Sci. 74: 2889-2893, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07104-03 LMCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Nutritional and Pharmacological Regimens for Potentiation of Chemotherapeutic Activities

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marco Rabinovitz, Ph.D.	Research Chemist	LMCB	NCI
OTHER:	David T. Vistica, Ph.D.	Staff Fellow	LMCB	NCI
	Anton Naujokaitis, M.D.	Senior Staff Fellow	LMCB	NCI
	Yoshimasa Uehara, Ph.D.	Visiting Fellow	LMCB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Molecular Biology and Methods Development

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.7	3.5	2.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to develop procedures which will potentiate the activity of current and novel cancer chemotherapeutic agents. Binding, transport, cell membrane composition and agent detoxification are modified in an attempt to direct the active agent to susceptible tumor cells. Investigations are initiated in tissue culture and when applicable are extended to tumor bearing mice. Studies include (1) therapeutic interference with melphalan treatment by leucine and glutamine and its abolition by basic amino acids and by glutaminase:asparaginase, respectively and (2) differential competition for carrier-mediated transport of cytotoxic agents. In this approach, non-cytotoxic competitive substrates are used to discern the differences in structural requirements for transport between tumor cells and a sensitive host tissue such as the hematopoietic precursor cells of the white cell series. Knowledge of such differences is used both to develop bone marrow protective agents and to synthesize cytotoxic agents with greater specificity for the tumor cell.

Project Description:

A. Basis for continuation, development and termination of project elements

1. Membrane Modification Studies: Attempts were made to develop an *in vivo* model for our earlier observation *in vitro* that tumor cells treated with ergosterol were 1000 x more sensitive to the lethal action of amphotericin-B than untreated cells. All such attempts were unsuccessful, and in keeping with our policy of using tissue culture studies only as a basis for development of rational *in vivo* protocols, this approach was terminated.

2. Serum Binding and Displacement Studies: Studies with phenylbutazone indicated a correlation between its ability to displace chlorambucil *in vitro* from bound form in serum and its ability to potentiate the activity of chlorambucil *in vivo*. Under two treatment schedules, the dose of chlorambucil for equivalent ILS of tumor-bearing mice was reduced, but the ILS itself was not increased. The data indicated identical increases in both antitumor activity and host toxicity by displaced chlorambucil, and since no therapeutic advantage was observed upon displacement of a bound antitumor agent, this area of investigation was terminated.

3. Carrier-mediated Transport in Cytotoxicity and Therapy: Inhibition of melphalan transport and cytotoxicity by leucine *in vitro* can be correlated with therapeutic interference by leucine *in vivo*. Both cytotoxic and therapeutic interference can be abolished by the use of appropriate basic amino acids of the arginine homologous series. Glutamine also inhibits melphalan uptake and cytotoxicity *in vitro*. This effect is readily overcome by glutaminase:asparaginase, and this enzyme markedly increases the number of 60 day survivors when administered to tumor-bearing mice prior to melphalan treatment. In view of these close correlations between *in vitro* and *in vivo* observations, this area has been expanded and is the basis for clinical recommendations.

A survey of potential cytotoxic agents whose transport may be carrier-mediated disclosed that, in L1210 cells, contrary to current belief, uracil mustard was not transported by a purine and/or a pyrimidine transport system and that chlorozoticin was not transported by the glucose transport system. Showdomycin, a nucleoside antibiotic with cytotoxic properties due to the reactivity toward sulfhydryl groups of its maleimide moiety, was cytotoxic to L1210 cells. The cells could be protected by a variety of purine and pyrimidine nucleosides. Mechlorethamine (HN2) was highly cytotoxic and in accord with literature reports this cytotoxicity could be reduced by choline.

4. Differential Competition for Transport of Cytotoxic Agents, An Approach to Selectivity in Cancer Chemotherapy: A principal concern in cancer chemotherapy is the protection of sensitive host tissues, such as the hematopoietic progenitor cells of the bone marrow, from the action of cytotoxic agents. We have therefore developed and applied an *in vitro* cloning assay for evaluating the structural requirements of substrates for protection

against carrier-mediated cytotoxicity to the granulocyte-macrophage progenitor cells of mouse bone marrow. Differences in structural requirements for protection against L1210 cells and bone marrow cells were noted and are being developed in either of two directions. If the analog serving as competitive substrate is more protective to bone marrow cells, then its co-administration in vivo with the cytotoxic agent should lead to increased chemotherapeutic specificity. Conversely, if structural features render a compound more protective to L1210 cells, then incorporation of such features into a cytotoxic agent could make it more selective against the tumor cells. Cytidine was found to be twice as protective against showdomycin for bone marrow cells than for L1210 cells and conversely, homoleucine was twice as protective against melphalan for L1210 cells than for bone marrow cells. The elaboration of these approaches to applied chemotherapy and drug development will remain the principal thrust of our unit for the near future.

Publications:

Schiffman, F.J., Fisher, J.M., and Rabinovitz, M.: Serum Displacing Agents in Cancer Chemotherapy. Cancer Treatment Reports 61: 1407-1409, 1977.

Schiffman, F.J., Uehara, Y., Fisher, J.M. and Rabinovitz, M.: Potentiation of Chlorambucil Activity by Phenylbutazone. Cancer Letters (in press) 1978.

Schiffman, F.J. and Klein, I.: Rapid induction of Amphotericin B sensitivity in L1210 leukemia cells by liposomes containing ergosterol. Nature 269: 65-66, 1977.

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Vistica, D.R., Toal, J.N. and Rabinovitz, M.: Amino Acid Conferred Protection Against Melphalan: Interference with Leucine Protection of Melphalan Cytotoxicity by the Basic Amino Molecular Acids in Cultured Murine L1210 Leukemia Cells. Molecular Pharmacology (in press), 1978.

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SUMMARY REPORT OF THE LABORATORY OF TOXICOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1977 to September 30, 1978

I. Introduction

The Laboratory of Toxicology continues to try to honor its dual responsibilities for conducting basic research and for the supervision of large animal toxicologic studies of antineoplastic agents prior to their introduction into man. This year the Division recompeted its Prime Contract, wherein the responsibilities for the large animal toxicologic studies are shared by the Prime Contractor (Battelle Memorial Institute) and the laboratory. Undoubtedly, the Prime Contract has made available more time for the in-house staff to properly pursue some important aspects of research into toxicological mechanisms of action of antineoplastic agents.

For the past fiscal year, the laboratory committed itself to three major goals: 1) recruitment of high quality replacements for the individuals who recently left LT; 2) continued improvement of the in-house basic research; and 3) to cooperate with the Prime Contractor in maintaining the excellent technical momentum achieved during the first four contract years. It was felt that with the continuing limitations of staff, it would be unrealistic to project additional goals.

Partial success was achieved regarding the first goal, since two of the three available senior positions have been filled. This was accomplished only after a very vigorous recruitment which took more than one year, involved seeing more than 60 applications and included interviewing about one-third of these candidates. Because this was the first occasion in the history of LT that we have had the opportunity to recruit at this level, it was our first encounter with the severe federal salary restrictions which exist at the senior professional levels in extreme shortage categories, in this case pathology. Thus we found that once we identified a professional with suitable credentials as a proven investigator in the field, he invariably had competing offers which averaged twice the present top GS salary! As a compromise, we have hired younger investigators, who, though as yet unproven, show considerable promise. Specifically, we sought to heed the recommendations which followed our recent peer review site visit, i.e., to acquire individuals trained in areas of pathology who could follow morphologic leads to a molecular subcellular level. We have yet to fill the third position, that of a person with veterinary/morphology training. This subspecialty is also a shortage category since the private sector needs such individuals to assist in complying with the Toxic Substance Control Act and the Good Laboratory Practice Regulations. Our program and in-house research needs resemble those in the private sector since the Drug Developmental Program obligations, vis-a-vis FDA, are quite similar. Irregardless of whether a xenobiotic is a pesticide, an industrial chemical or a drug, its effects on individuals and the environment are ultimately and most definitively assessed by histopathologic evaluation of organisms which have been exposed to the xenobiotic in question.

The turnover in the past year caused by both professional and support personnel transfers, primarily from the Pathology section, amounted to about 50% of the LT staff. Despite this potentially devastating fact, we were able to sustain momentum in several specific technical areas and in the general area of quality and quantity of research publications. Regarding this latter point, one Section (Drug Interactions) published nearly as many papers this year as did the entire LT last year. In addition to excellent scientific design of experiments, this Section's accomplishments were definitely aided by the reallocation of space (1 module) to them.

In the highly important area of basic toxicology, the LT has continued to develop and support the application of target organ testing. Thus, in order to employ a small animal for testing adriamycin analogs for cardiotoxicity, a rat model (Mettler et al., Cancer Research, 37: 2705, 1977) was offered. In the rat, congestive heart failure (CHF) develops as it does in adriamycin-treated patients. Although conserving quantities of expensive drugs, this system apparently is too slow (i.e., 3-4 months) to give synthetic chemists the rapid turn around time they need to develop analogs. Vigorous efforts to develop a mouse acute model continue in LT but so far have not proved to be either sufficiently reproducible or to correlate with the development of CHF. While following our mandate to develop an acute model (which is unlikely to predict for the chronic effects of this class of cardiotoxic antineoplastic agents), we favor the rabbit, rat, and mouse chronic models, which while taking longer to accomplish, are more likely to mimic the pathogenesis of the disease as it occurs in humans. In other words it is scientifically more likely that the chronic model will be relevant while the odds are that the acute model is likely to be scientifically irrelevant.

In addition to the cardiac target organ model in the rabbit and rat, LT has developed a nephrotoxicity model in the rat (Ward et al., CTR 60: 675, 1976). The value of these studies is now being recognized as it is being employed by our Prime Contractor, in identifying 2 out of a dozen platinum containing analogs which cause less renal tubular toxicity than does the parent drug. The specific site in the kidney affected is the same in the rat and in humans.

In a third target model (Sikic, et al., Cancer Research 38: 787, 1978) we have developed a bleomycin pneumotoxicity model employing morphometric and biochemical parameters in the mouse. This model has already proven its ability to differentiate between the toxicities of three different dosage schedules of bleomycin. Frequent injections of small individual doses of this drug is therapeutically more effective than high intermittent doses; the pulmonary toxicity is the same. Other studies using this model to find less pneumotoxic bleomycin analogs are currently ongoing.

Other topics covered in this portion of the annual report in previous years included the following: 1) lack of staff growth commensurate with increases in contract and research obligations of LT; 2) the staff efforts required to sustain and evaluate the performance of the Prime Contract (because it is of the award-fee type); 3) a caution against complacency regarding the excellent technical performance of the Prime Contract (even though they accomplished the history-making task of completing a full protocol study on PALA in about one-half the usual time); and 4) the need for up-to-date bench scientists to guide our contract operations because of the dynamic nature of contemporary toxicologic testing (compared with the relatively well-established protocols used for experimental chemotherapy screening). The reader is referred to previous years' annual reports for further details in these areas. These items are mentioned again because they continue to be areas of considerable concern to us since little has been done to resolve these problems.

II. Internal Research Programs

The independent and joint projects of the senior staff members are primarily directed toward attempting to understand the actions of antineoplastic agents, especially those actions which manifest themselves as some form of toxicity. It is only through an understanding of such actions that we will be able to advise clinicians intelligently as to what toxicity to expect, when toxicity does occur, and what steps may be taken to reverse it.

The practice of modern toxicology is really impossible to separate from pharmacology. Obviously, the properties of drugs and biologic membranes which bring a drug to a site of pharmacologic action, can also induce toxic responses. Our general approach is quite broad because we study drugs through a series of complex processes that end in a potential lesion. Thus, we are interested in the drug during the exposure phase (bioavailability), the pharmacokinetic phase (absorption, distribution, metabolism and excretion), the pharmacodynamic phase (therapeutic) and finally, the toxicodynamic phases.

As far as the science of toxicology is concerned, those of us who work with antineoplastic agents continue to recognize that we have an extra obligation to our colleagues working with other classes of xenobiotics. This is because oncolytic agents include some of the most toxic substances to which animals and man are intentionally exposed. Also, while the overall ability of animals to predict for human toxicity is poor, this is largely the result of the inadequacies of the human toxicologic data. For example, those working with pesticides must usually settle for the poorly quantified occupational exposures as indices of human toxicity. Since we obtain both animal and human toxicity information on a qualitative and quantitative basis, we are in a better position to assess the animal to human correlations of such data. In general, animal tests of our drugs have been successful, as is attested to by the relatively small number of serious toxic reactions which were not anticipated as a result of animal studies. More specifically, in a very thorough survey completed this past year, several new points emerged: 1) out of 120 clinically important target organ toxicities for 44 anticancer drugs, large animals (dogs and monkeys) predicted (true positives) 83% of these; 2) most of the predictiveness, 81% came from the canine species thereby questioning the value of continuing to use primates, which have become increasingly scarce and expensive; 3) marked improvements (2-5 fold) have occurred since 1970 in the qualitative predictiveness of the following organ systems: hepatic, respiratory, nervous, urinary and injection site. These improvements are due to a combination of factors such as the following: 1) publication and adherence to a protocol [Prieur, et al., CCR, 4 (3): 1, 1973]; 2) state-of-the-art changes (e.gs. inclusion of more clinical chemical tests and more complete histopathologic evaluation); 3) more astute cage-side observations on the part of subcontract staffs. While the quantitative data base is not as extensive as for the qualitative studies, quantitative toxicities comparing dogs and mice showed that the prediction of clinical starting doses from data generated from either species alone, carried with it a 12-14% risk of encountering serious toxicity during the first stages of Phase I clinical trials. On the other hand, if the results of the more sensitive species between the dogs and mice were considered, no examples of serious Phase I toxicity would have occurred at the initial starting doses.

The major mission of the Biochemistry Section is to characterize the biochemical basis for the toxicity of certain antineoplastic agents. A secondary role of this Section has been to develop and apply sensitive methods for the detection of enzymes and substrates through the use of radiometric techniques. In very complete studies of L-alanosine (NSC-153,353) and PALA (NSC-224,131), agents which have recently entered clinical trials, both areas of expertise have been utilized. Similar in-depth biochemical studies are in progress with isoxazoleacetic acid analog (NSC-163,501) which is presently under preclinical development.

This Section is involved with the adriamycin-vitamin E saga, and has not been able to consistently reproduce the results of Myers, et al., (CTR, 60: 961, 1976) in mice. With the passage of time, the Biochemistry Section has moved away from its search for inhibitors of certain target enzymes important to the economy of the tumor cell and has begun to focus instead on the target enzymes themselves. This change in polarity has manifested itself in emphasis on three projects during the last year: the first involves an inquiry into the enzymologic, and pharmacologic basis for resistance of select neoplastic cells to PALA (N-Phosphonacetyl-L-Aspartic Acid); the second involves an examination of the enzymatic basis for the toxicity of alanosine to tumor cells and normal tissues; while the third entails quantification of those factors present in extracts of human breast and colon tumors which are responsible for converting 5-Fluorouracil to its several cytotoxic forms. Each of these areas have much to do with why drugs fail, which, unfortunately, is most often the case in human trials.

It is now well documented that PALA acts by restricting the availability of the pyrimidines through its inhibition of L-aspartic acid transcarbamylase (ATCase). In this context it was not unreasonable to expect tumors deficient in this target enzyme to be most responsive to PALA. During the past year therefore, the specific activity of ATCase has been measured in extracts of

12 rodent neoplasms. It was observed that all tumors in whose cytoplasm the specific activity of this enzyme was below ∿0.2 μmoles/mg/hr were sensitive to PALA in therapeutic trials, whereas those with levels greater than this were resistant. It can be concluded that there is an enzymologic basis to the responsiveness of at least some rodent tumors to PALA. At present, analogous measurements are being carried out on human blood and biopsies of human tumors. In the small number of cases so far examined, the specific activity in both of these sources is lower than what had been seen in comparable mouse systems; this may indicate that the therapeutic or toxic actions of PALA in man should parallel those of the sensitive rodent tumors.

In studies of the enzymologic basis for the activity of L-alanosine, it was shown that in vivo this drug restricts the availability of AMP, and thus of ATP and dATP. This restriction has been traced back to an inhibition of adenylosuccinate synthetase which, in this chemotherapeutic case, can be considered the target enzyme. In vitro, however, alanosine, per se, does not inhibit its target at pharmacologically realistic concentrations. The causes of this paradox have been under examination in the Biochemistry Section over the past several years. These studies have uncovered a complicated but fascinating enzymologic system for anabolizing alanosine, and then for destroying the anabolite.

Briefly, it has been observed that alanosine is enzymatically condensed in vivo with a fragment of the nascent purine ring to yield an adduct (alanosyl AICAR) which is capable of extremely potent inhibition of adenylosuccinic acid synthetase in vivo and in vitro. This adduct is susceptible to destruction by three cellular enzymes: alkaline phosphatase, 5' nucleotidase and, most importantly, adenylosuccinic acid lyase. Thus, an enzymologic triangle is at work here: the anabolizing enzyme, the catabolizing enzyme and the target enzyme. Measurements of all three enzymes have been made in 3 pairs of rodent tumors, one of each pair being resistant to alanosine. The specific activities of the anabolizing enzyme and the target were not significantly different in these cases, but the lyase which catabolizes the active molecule formed in vivo from alanosine, was paradoxically depressed in the resistant lines. This result is puzzling but may be due to the necessity of maintaining the resistant state through daily administration of alanosine. Analogous measurements of the three enzymes reported here were carried out in normal organs of the dog with an eye to correlating enzymology and toxicology. Thus, for example, it was found that duodenum, which is susceptible to inflammation by alanosine, was exceptionally deficient in the lyase. This deficiency may have etiologic importance, therefore.

Roughly 90% of human colon and breast adenocarcinomas do not respond to 5-FU and therefore, there is a need to develop predictive enzymologic methods to screen out those cases where marked resistance is present. Using extracts of human tumors, a five-pronged approach was taken, in vitro, using the following measurements: 1) the specific activities of the several phosphorylases and transferases which convey the ribose or deoxyribose moiety to 5-FU; 2) the kinases which phosphorylate the resultant nucleosides to the level of the mono-, di-, and triphosphate; 3) the ability of fortified extracts to generate FUTP and FdUTP in vitro; inhibition of RNA polymerase is used to this end; 4) the ability of fortified extracts to generate FdUMP in vitro; inhibition of Thymidilate synthetase is used to this end; 5) the phosphatases and nucleotidases which decompose FdUMP and FUTP. This comprehensive approach has so far been applied to murine leukemia P388 and P388 resistant to 5-FU, as well as to a panel of 15 human adenocarcinomas. In the murine model, it has been observed that there is little or no deficiency in enzymes conveying the sugar and one phosphate to 5-FU, but that the higher order kinases are markedly deficient in P388/FU This suggests deletion of a specific necleotide kinase. In the samples from women, the one consistent finding has been that fortified extracts of human mammary adenocarcinomas generate far more FdUMP than analogous extracts of murine tumors. This difference ought to be exploitable.

The primary efforts in the Comparative Pathology Section have continued in the area of adriamycin (ADR) induced cardiotoxicity, the major dose-limiting toxicity in the clinic. Having established the rabbit as a reproducible model for assessing the cardiomyopathy, efforts were turned toward developing smaller animal models. Current investigations were designed to determine if ADR would induce a significant and reproducible cardiotoxic syndrome in the rat and/or the mouse. The majority of ADR-treated rats developed cardiomyopathy 3 to 23 weeks after the last injection. Gross evidence of congestive heart failure was also observed. The results of this study suggest that the rat model should be further developed for large scale cardiotoxicity testing. In related studies, the Myers' hypothesis regarding the protective efforts of vitamin E vs the cardiotoxicity of adriamycin was tested in the rat and rabbit. Intraperitoneal administration of vitamin E offered no protection to the toxicity of adriamycin in the rat and the rabbit in contradiction to results reported for mice. In fact, the results in these former species not only showed potentiation of toxicity, but provided clues regarding how the vitamin may be exerting its effects in mice. In the rat and rabbit, severe peritonitis invariably occurred, thus suggesting that the sparing action of vitamin E in mice may be via protection against the chemical peritonitis or some other kind of local toxicity caused by intraperitoneal administration of adriamycin.

This Section has published three papers establishing and utilizing the Fisher rat model for prediction of Pt-compound nephrotoxicity. It was found that both BUN values and histopathologic evaluation of renal lesions were necessary to determine the type and severity of nephrotoxicity. The model has proved to be useful in testing potential antidotes, (e.g., furosemide diuresis) to Pt-induced toxicity.

In a computer-assisted evaluation of adverse tissue effects of antineoplastic agents as revealed by postmortem examinations of tissues from clinical center patients, we studied this important aspect of animal-human correlations. The results of 600 autopsy reports from patients treated for neoplasia by chemotherapy, irradiation, surgery or combinations of 2 or all of the modalities have been entered into computer storage and programmed for ease of retrieval. Of these 600 patients, 324 (54%) are males and 276 (46%) are females. They ranged in age from 2 to 90 years with nearly half being between the ages of 31 and 60. Thirty-seven were 10 years or younger and 28 were above the age of 70. Most patients had either leukemia or lymphoma. One hundred twenty (20%) had acute leukemia (ALL or AML) and 72 (12%) had chronic leukemia (CML or CLL). Eighty-three (14%) had non-Hodgkin's lymphoma and 59 (10%) had Hodgkin's lymphoma. Sixty-eight had adenocarcinoma, 47 squamous cell carcinoma, 36 melanoma and 30 various sarcomas. The remainder were divided into rarer neoplasias such as germ cell, brain and plasma cell tumors. Of the 600 autopsies, 464 were performed in the NCI laboratory and 136 were reviews of submitted slides from autopsies done on NCI patients who died elsewhere. Five hundred forty-five (91%) were treated with antineoplastic agents, 306 (51%) with irradiation and 34 (6%) by immunotherapeutic regimens such as intracutaneous BCG or derivatives thereof.

Information concerning at least 46 drugs was stored. These were mostly antineoplastic agents but also included selected antibiotics, allopurinol and INH. Twenty-three drugs were given to at least 25 patients. The 6 drugs used most frequently and listed in order of frequency were: corticosteroids (326, 54%); vincristine (322, 53%); cyclophosphamide (247, 41%); methotrexate (196, 33%); ara-C (156, 26%); and, 6 MP (121, 20%). Charts have been made which list each patient, diagnosis, and drugs received. Using the order in the Systemized Nomenclature of Pathology, the principal morphologic changes were: inflammation (409, 68%); fibrosis (367, 61%); atrophy (357, 60%); necrosis (279, 47%); deposition of materials such as fat, amyloid and calcium (246, 41%); and, acute inflammation (242, 40%). The future aims are to relate specific changes in each organ with the use of various drugs and combinations of drugs and with the primary neoplastic processes.

The major thrust of the Drug Interactions Section is the study of "drug-drug interactions". This is particularly important since patients with neoplastic disease frequently receive a multitude of drugs which may alter the absorption, binding, distribution, excretion, metabolism or pharmacokinetics of the anticancer agent. Such activities are important in defining which parameters lessen drug toxicity, e.g., microsomal induction, and in understanding factors increasing toxicity. These studies have become increasingly important because of the growing evidence that drug biotransformation, leading to irreversible tissue-binding, is a major parameter associated with toxicity. An increasing number of literature citations offer evidence that relatively inactive drugs can be activated in vivo to proximate toxicants. Over the past several years, this Section has studied hepatic, pulmonary and renal drug metabolism and now has the foundation upon which the LT is building a major five-year-program related to lung cancer.

In a related category, drug-diet interactions also are possible and have been studied in this Section. A recent review of the nutritional status of patients with cancer (Postgrad. Med. 61: 97, 1977) pointed out that 15% have significant anorexia at the time of disease recurrence or metatases and that with further progression of disease, anorexia increases. This reference further states that in such patients there is "frequent development of protein-calorie malnutrition, vitamins and other deficiencies". Thus, after completion of studies of vitamin C interactions, this group has become involved with vitamin A. In experiments on rats in different stages of avitaminosis A, it was found that most drug metabolizing activities remained equal to those seen in normal rats, but glutathione S-aryl transferase activity increased two-fold after a month or more on the deficient diet. Since many anticancer drugs are handled by this pathway, such an increased rate of metabolism cannot be ignored.

As part of our continuing studies to understand the mechanisms by which antineoplastic drugs elicit toxicity we have investigated the effects of another vitamin, vitamin E (α-tocopherol), on the pharmacokinetics, disposition, metabolism and lethality of adriamycin in CDF_1 mice. Animals were treated with either vitamin E, olive oil or saline solution, ip, 24 hours prior to ^{14}C-adriamycin administration, 15 mg/kg, also ip. Vitamin E and olive oil caused similar increases in blood and tissue levels of ^{14}C-adriamycin-derived radioactivity. Blood levels of adriamycin-derived radioactivity in animals pretreated with olive oil or vitamin E were approximately twice those of saline controls between 3 and 15 min after injection, and at 60 min, levels of radioactivity in liver, kidney, lung, and heart were significantly higher in the vitamin E and olive oil groups than in saline controls. A number of pharmacokinetic parameters for adriamycin were increased, most notably resulting in greater c x t values in the vitamin and oil treated groups. Profiles of metabolites in heart and other principal organs revealed no significant difference in the metabolism of adriamycin in any of these different groups of mice. Typical of the results now emerging in the vitamin E-adriamycin saga, animals treated as described above lost weight at similar rates with maximum losses (25%) in the survivors at 7-14 days. Fourteen days after adriamycin treatment there was 50% mortality in both the olive oil and saline groups, but only 5% mortality in the vitamin E group. However, by 28 days, 30% of the animals in the vitamin E group were dead compared to 60% in the other 2 groups, and at 60 days, mortality was 80 ± 5% in all 3 groups. Our studies suggest that the major effect of vitamin E on the lethal toxicity of adriamycin is to delay rather than prevent it. The distribution studies are consistent with ultimate toxicity data in that the former studies predicted, perhaps enhanced, but certainly did not diminish cardiotoxicity, as evidenced by increased c x t values in the heart.

A second major area of involvement of this section stems from its interests and expertise in the area of pharmacologic disposition and toxicodynamic aspects of the lung. Pulmonary neoplasms are the leading cause of cancer deaths among Americans (about 17% of all tumors in males), and, the relative incidence of lung cancer deaths among males has steadily increased since before 1940 with no hint of a plateau in sight. Only about 10% of the patients survive 3 or more years. Progress in evolving new chemotherapy, either single agents or combinations, or combined modality therapy for malignant pulmonary tumors has been distressingly slow.

In considering the pharmcologic approach, evidence has been presented over the last decade that basic drugs (pKa >8.5) are concentrated and retained by the lungs of mammalian species. However, recent work in this section suggests that the accumulation of such xenobiotics is more complicated than suggested by previous data. In investigations of the pulmonary retention of a model basic drug (imipramine) in several species, we found both species and dose dependency. Thus, in vivo, rats and rabbits, but not mice and guinea pigs, exhibited significant accumulation of radioactivity in the lungs relative to other tissues.

In a related project the schedule dependency of bleomycin therapy and pneumotoxicity was assessed in Lewis Lung tumor-bearing mice. The starting point of these studies was suggested by references in the clinical literature that continuous intravenous infusion of bleomycin, or more frequent injections of lower doses, may result in an improved therapeutic index as compared to conventional weekly treatment by iv bolus. Tumor-bearing mice were treated with three schedules of bleomycin: 10 mg/kg twice weekly versus 1 mg/kg and 2 mg/kg ten times weekly. For the latter two schedules, ILS values were significantly superior to controls. However, the more intermittent schedule, 10 mg/kg twice weekly, did not result in significantly increased survival over controls. There was no significant difference in lung toxicity produced by 10 mg/kg twice weekly for four weeks versus 2 mg/kg ten times weekly for four weeks. Pneumotoxicity was measured by total lung collagen content and the following morphometric parameters: number of intraalveolar macrophages and leukocytes, total pulmonary cell count, mean alveolar wall thickness, and percent consolidation of lung parenchyma. At equivalent total doses of bleomycin, more frequent injection of small individual doses of drug is more effective in treatment of Lewis Lung carcinoma than high, more intermittent doses, while the pulmonary toxicity of the two schedules is approximately the same.

Further experiments have compared these schedules of bleomycin injection with continuous infusion of bleomycin subcutaneously (sc) in mice via the Alzet^{R} minipump. Continuous infusion was significantly superior to equivalent doses of bleomycin administered by intermittent injection in reducing the size of Lewis Lung carcinoma. Effects of these schedules upon pulmonary toxicity were compared using whole lung collagen content. A total dose of 40 mg/kg of bleomycin, sc, produced significant increases in lung hydroxyproline levels compared to saline controls when the bleomycin was administered by injection twice or ten times weekly, but no increase in this index of pneumotoxicity was seen when the drug was given as a continuous infusion for seven days. Thus, continuous infusion reduced the pulmonary toxicity of bleomycin and increased therapeutic efficacy against Lewis Lung carcinoma in mice. Since randomized comparisons of continuous infusion versus intermittent injection of bleomycin have not been done in man, this is the first rigorously controlled experimental evidence demonstrating that continuous infusion of bleomycin actually does improve the therapeutic index of the drug.

In continuation studies to provide basic data for the intraperitoneal chemotherapy of cancer, the absorption characteristics of 15 antineoplastic agents were determined; their absorption ranged from 9% for L-asparaginase to 91% for hexamethylemelamine. A correlation was made to their respective molecular weight, pKa and K values. From these results, recommendations were made as to which agents (i.e., those with minimal absorption) should be employed in this adjunct mode of therapy for ovarian carcinoma.

Further experimental work has shown that the transport characteristics of the peritoneal membrane apparently do not change during an 8 hour course of therapy. Eight hour absorption of both 5-FU and adriamycin followed rates of absorption predicted by the 1 hour data, and organ distribution was found to be similar to that demonstrated after one hour of exposure. Fluid transport out of the peritoneal cavity was found to be highly dependent on the vehicle. In contrast to rapid decreases in volume when 0.85% NaCl is used, Inpersol (1.5% dextrose solution utilized in clinical peritoneal dialysis) results in intraperitoneal volumes that are sustained or increased by 20% over an 8 hour time course. This change in fluid shift, however, did not significantly alter the absorption of 5-FU or adriamycin.

III. Contract Program

We have now completed four years of operation under the Prime Contract mechanism. The technical aspects of this program are working exceedingly well. During the first contract year, the Prime awarded two subcontracts, in the second year three additional subcontracts, and two more in the third year. The major goal of the Prime Contract is the continuation of protocol toxicity studies in large animals. The Prime has provided the Division with a mechanism for rapid completion of protocol studies 40% faster than previously, with cost savings of approximately 20%. During the past few contract years one-half the projected number of protocol drugs were available. The Prime has made more research time available to the LT scientific staff to pioneer methodology which can eventually be taken over by the Prime. For example, a rat model to assess the relative nephrotoxicity of a series of platinum analogues was developed by the LT and implemented (through the Prime Contractor) at a subcontracting laboratory. Similarly, methodology to assess peritoneal toxicity of antineoplastic agents and pneumotoxicity of bleomycin analogs, both by procedures developed in-house, are nearing the stage where the Prime can take them over to process larger numbers of compounds than could be handled by LT. This type of a developmental and field-testing role is imperative in an area changing as rapidly as is modern toxicology.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03518-10 LT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Pharmacologic Interruption of the Metabolism of the Dicarboxylic Amino Acids and their Amides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

David A. Cooney, Senior Surgeon, BS, LT, DTP, DCT, NCI
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LAB/BRANCH
Laboratory of Toxicology

SECTION
Biochemistry Section

INSTITUTE AND LOCATION
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TOTAL MANYEARS: 4	PROFESSIONAL: 3	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The biochemical mechanisms by which PALA, L-alanosine and 5-fluorouracil produce toxicity were examined: For PALA, six mechanisms were explored: differences in transport, in enzyme kinetics, in catabolism of PALA, in the activity of the enzymes of pyrimidine salvage, in the rate of pyrimidine catabolism, and in the specific activity of L-aspartate transcarbamylase in vivo. The best correlation was observed with the last parameter: tumors and tissues with low levels of the transcarbamylase were susceptible to PALA. 2) In the case of L-Alanosine, the rate at which the effector metabolite is formed and destroyed in a given tissue or tumor was correlated with susceptibility to the drug. In two lines of tumor cells, one sensitive and one resistant to Alanosine, no marked difference in the specific activity of the anabolizing enzyme, (SAICAR kinosynthase) was observed. However, the catabolizing enzyme, adenylosuccinate lyase was elevated in the two sensitive tumors. 3) In the case of 5-FU, the ability of extracts of cancers to form and destroy the inhibitory species, FUTP and FdUMP, has been examined in vitro. Those tumors richest in enzymes phosphoralating FU and deficient in the phosphotases which destroy the resultant anabolite were most susceptible.

Objectives:

To characterize the biochemical basis for the toxicity of select oncolytic drugs and, where possible, to devise and develop antidotal measures.

Methods Employed:

Standard and experimental enzymatic, biochemical and analytical techniques have been utilized.

Major Findings

During the past year, the Biochemistry Section of the Laboratory of Toxicology has directed its attention to the mechanisms of action and toxicity of 2 new agents and two established oncolytic drugs. These are PALA, (NSC-224,131) L-Alanosine, (NSC-153,353), 5-Fluorouracil (NSC-19,893) and L-asparaginase (NSC-109,229). Work with each of these will be considered.

I. Studies with L-Aspartic Acid Analogs

A. Studies with PALA [L-Aspartic acid, N-(phosphonacetyl)-, tetrasodium salt, NSC 224,131]. PALA inhibits L-aspartate transcarbamylase (ATCase), the second enzyme in pyrimidine biosynthesis. The drug exhibits potent antitumor activity against some murine solid tumors, and has entered Phase I Clinical Trials. To permit pharmacokinetic studies of PALA in humans, we have developed an enzymatic-radiometric method for the estimation of this agent in plasma, urine, tissues and tumors. Samples are homogenized in a medium containing 30% dimethylsulfoxide. Tightly bound PALA is quantitatively detached from its target enzyme, ATCase, by heating at 95° for 5 min. Denatured proteins are removed by centrifugation. PALA, in the supernatant fluid is quantitated by exposing intact splenic ATCase to representative aliquots or subdilutions of the resultant supernatant in the presence of L-[4-^{14}C]aspartic acid and carbamyl phosphate. After a 30 min. incubation at 37°, unreacted L-[4-^{14}C]aspartic acid is dissipated enzymatically, and newly formed [4-^{14}C]carbamyl-L-aspartic acid is quantitated by scintillation spectrometry. The percent inhibition of ATCase responds in a linear way to the logarithm of the concentration of PALA between 0.1 and 1.0 μM. The PALA concentration of the test sample is determined indirectly by matching the percent inhibition caused by the unknown to the inhibition caused by a known series of standard concentrations of PALA over the linear range. This assay is sensitive, adequately reproducible despite the use of an unpurified enzyme, and notably facile. Using this assay, the disposition of PALA has been examined in four patients treated with the drug at a dose of 100-200 mg/M^2. In 3 of these patients the half-life of PALA in plasma ranged from 1.5 to 3.7 hours. In one patient, 65% of the injectate was accounted for in the urine.

B. Mechanism of PALA Toxicity. To explain the mechanism of sensitivity and resistance of rodent tumors to PALA, several hypotheses were explored: 1) Sensitivity is a function of low specific activity (S.A.) of the target enzyme, ATCase, in sensitive as opposed to resistant tumors. In 8 PALA-sensitive and 6 PALA resistant tumors, the mean (± S.D.) specific activity of ATCase was 0.2097 ± 0.0899 and 0.4978 ± 0.0934 μmoles of carbamyl-L-aspartate formed/mg protein/hr, respectively ($p<0.001$), indicating a significantly higher activity of ATCase in resistant than sensitive tumors. 2) Sensitivity is a function of a lower K_i of PALA for the enzyme from sensitive than resistant tumors, or to other unique kinetic features. This hypothesis was nullified since the K_i of PALA and the K_m for the substrates carbamyl phosphate and L-aspartic acid, towards ATCase from prototypical sensitive and resistant tumors were closely comparable. 3) Resistance to PALA is a function of rapid restitution of ATCase in tumors refractory to this agent. This hypothesis was nullified inasmuch as the rate of reappearance of this target enzyme after single doses of PALA causing 90% inhibition of ATCase was not significantly different in resistant versus sensitive tumors. 4) PALA is transported into sensitive tumors at a rate which is greater than the rate seen with resistant counterparts; irrespective of sensitivity or resistance, cells exposed to 250 μM PALA *in vitro*, transported only 1.0% of the drug over a period of 4 hrs. 5) PALA is catabolized in tumors resistant to this agent; no evidence for this could be adduced using sensitive chromatographic techniques. 6) Tumors resistant to PALA could transport preformed pyrimidines from the extracellular space with greater affinity or a greater maximal velocity. Carrier mediated transport studies with uridine indicated a somewhat higher V_{max} in sensitive than resistant cells. 7) Tumors sensitive to PALA are deficient in the enzymes of pyrimidine salvage. Our studies indicated that pyrimidine nucleoside kinase levels are significantly higher in resistant than in sensitive cells; this is not, however, a general phenomenon.

C. Influence of PALA on carbamyl phosphate and L-aspartic acid pools. Since ATCase transfers the carbamyl moiety of carbamyl phosphate to L-aspartic acid to form carbamyl-L-aspartate, a study of the pool sizes of carbamyl phosphate and L-aspartic acid in sensitive and resistant neoplasms was undertaken to further characterize metabolic effects of the drug. Carbamyl phosphate is highly unstable; therefore, suitable methods for its extraction and measurement were examined. The following technique, which is a substantial modification of that presented in last year's annual report, proved to be feasible. Organs of mice are flash-frozen between blocks of solid CO_2 and then homogenized in acetone at 25°. Aliquots of the homogenate are transferred into Eppendorf test tubes, centrifuged, acetone is decanted and the pellet is resuspended in ice-water. The resultant slurry is recentrifuged and aliquots of the aqueous supernatant are dispensed into Eppendorf vessels resting on a bed of dry ice. L-[4-^{14}C]aspartic acid and purified ATCase from *Escherichia coli* are added and the mixtures incubated at 37° for 10 min. Any unreacted L-aspartic acid is dissipated enzymatically and the residue counted by scintillation spectrometry. This method is sensitive enough to measure 0.1 nmole of carbamyl phosphate. To verify that the anticipated product was being formed, reaction mixtures were scaled up 20-fold and deproteinized; carbamyl-L-aspartate then was analyzed on a column of Hamilton HAx4 resin, using the JEOL amino acid analyzer. Carbamyl-L-aspartate elutes at 56 minutes compared to L-aspartic acid which elutes at 18 minutes. No carbamyl-L-aspartate was formed when samples containing carbamyl phosphate were pretreated: 1) at 95° for 1 min; 2) with alkaline phosphatase; or 3), with L-ornithine and L-ornithine carbamyl transferase. It is therefore concluded that the method is measuring a thermolabile phosphate ester susceptible to condensation with L-ornithine, i.e., carbamyl phosphate.

Attempts were also made to measure the influence of PALA on carbamyl phosphate pools in liver. For this study, mice were injected intraperitoneally with 400 mg/kg of PALA or with saline, and sacrificed after 24 hrs; livers were then flash-frozen and analyzed for carbamyl phosphate. Preliminary results indicate the presence of 0.56 ± 0.18 and 0.57 ± 0.12 mmole of carbamyl phosphate/g of liver in PALA and saline-treated mice, respectively. Thus, at this time period, no accumulation of carbamyl phosphate was demonstrable. The significance of this finding lies in the fact that PALA competes with carbamyl phosphate for the catalytic center of L-aspartate transcarbamylase. If carbamyl phosphate is caused to accumulate, it will, in effect, dislodge PALA and shorten the span of its pharmacologic effects.

D. Mechanism of Action and Toxicity of L-alanosine. Administration of L-alanosine to mice results in inhibition of DNA synthesis and, after a lag of approximately 4 hours, restriction in the pool size of adenine nucleotides. This has lead to the inference that L-alanosine must inhibit adenylosuccinate synthetase in vivo. During the present year we have tested this inference: parenteral administration of therapeutic doses of L-alanosine does produce significant inhibition (∿ 50%) of adenylosuccinic acid synthetase in muscle. Adenylosuccinic acid lyase, the only other potential target for the antibiotic (see below), was unaffected. In vitro, L-alanosine is a comparatively feeble inhibitor of adenylosuccinic acid synthetase. Robert Hurlbert resolved this paradox, and explained that lag, by demonstrating that L-alanosine had to undergo complex anabolism before it would engender inhibition of adenylosuccinate synthetase; the K_i of the anabolite in question, "L-alanosyl aminoimidazolecarboxylic acid ribotide" is approximately 1 μM. This anabolite is a structural analog of the natural intermediate in purine biosynthesis, 5'-phosphoribosyl-4-(N-succinocarboxamide)-5-aminoimidazole, (SAICAR), whose decomposition is catalyzed by adenylosuccinic acid lyase. We reasoned that the anabolite of L-alanosine should be susceptible to decomposition, and therefore, inactivation, by this same enzyme. During the past year, then, we have prepared modest (μg) quantities of "L-alanosyl aminoimidazolecarboxylic acid ribotide", by the methodology given below, have partially purified adenylosuccinate lyase, have developed novel analytical methodology for measuring this enzyme, as well as the synthetase responsible for the generation of the aforementioned anabolite, and have tested the hypothesis that it is the balance between anabolizing and catabolizing enzymes which determines whether a tissue is susceptible to toxic damage or a tumor to lysis after administration of L-alanosine. Each of these areas will be considered in turn.

E. Preparation and Characterization of "L-Alanosyl Aminoimdazolecarboxylic Acid Ribotide". Incubation of DL-[1-^{14}C]alanosine with 5'-phosphoribosyl-5-aminoimidazole 4-carboxylic acid (AICOR) and ATP-Mg^{++} in the presence of a partially purified preparation of the condensing enzyme (the "kinosynthase") from chicken liver, led to the formation of a new nucleotide with a strong net negative charge at pH 2.72. This material was purified by high-resolution chromatography on Hamilton HAx4 resin and desalted on charcoal or DEAE-Sephadex using formic acid for elution. The ultraviolet spectrum of the eluate at pH 7.0 revealed two λ max: at 252 and 265 nmeters. This material yielded a characteristic, transitory salmon-pink chromogen with the Bratton and Marshall reagents; solutions of it with an absorbance of 0.35 x 10^{-4} at 265 nmeters still inhibited crude mouse muscle adenylosuccinate synthetase by 50%. In each of these respects, then, it is identical with the compound reported by Hurlbert, of whose work the present studies, therefore, supply independent confirmation.

F. Preparation of L-Alanosyl IMP. In view of the susceptibility of L-alanosine to condensation with AICOR, we reasoned that it might undergo an analogous condensation with IMP inasmuch as both of these steps are mechanistically homologous. Incubation mixtures were constituted, therefore, with DL-[1-^{14}C]alanosine IMP, GTP-$MgCl_2$ and a partially purified preparation of adenylosuccinate synthetase from rabbit muscle. A new radioactive nucleotide with a strong net negative charge at pH 2.72 was found and purified by successive chromatographies on Hamilton HAx4 resin and on charcoal or DEAE-Sephadex as described above. A material with identical chromatographic properties was formed when cold L-alanosine and radioactive IMP were used. It is therefore concluded that the new nucleotide results from the conjugation of L-alanosine with IMP to yield a compound structurally homologous to adenylosuccinic acid. When the L-alanosyl-IMP adduct was tested next as an inhibitor of adenylosuccinic acid synthetase, no inhibition was produced. Similarly, when the compound was examined as an inhibitor of adenylosuccinic acid lyase, no inhibition was produced. It is concluded that in mammalian systems L-alanosine can form an adduct with IMP but that it is not likely to be involved in the mechanism of action of the antibiotic.

G. Development of a New Analytical Methodology for Adenylosuccinate Lyase. When adenylosuccinic acid is metabolized to AMP and fumaric acid by the enzyme adenylosuccinic acid lyase (EC 4.3.2.2), a proton is lost from carbon 3 of the L-aspartate moiety. Advantage has been taken of this fact in the design of a facile assay for this enzyme: 2,3[^{3}H]adenylosuccinic acid is prepared from L-[2,3-^{3}H]aspartic acid and non-radiolabeled IMP using a partially purified preparation of adenylosuccinic acid synthetase from rabbit muscle. This substrate is purified by successive chromatography on Hamilton HAx4 resin with 0.015-0.225 M lithium citrate, pH 2.72 as eluent, then on DEAE-Sephadex, formate form, with a 0.025-3.0 M gradient of formic acid for elution. The product is lyophilized and reconstituted with 7 x 10^{-4} M cold adenylosuccinic acid; 5 μl aliquots are then incubated with 5 μl aliquots of enzyme. After an appropriate period, any tritium released to the medium as $[^{3}H]_2O$ is distilled at room temperature overnight into a 10 μl droplet of

100% KOH adherent to the lid of the reaction vessel. The lid is removed and [^{3}H] thereon is measured by scintillation spectrometry. The assay performed as prescribed, is facile, in that it permits the simultaneous estimation of lyase activity in a large battery of samples, is outstandingly accurate, and more sensitive than spectrophotometric methods.

H. Purification of Adenylosuccinic Acid Lyase. In order to test the hypothesis that the adduct of L-alanosine and AICOR was susceptible to decomposition, and therefore inactivation, by adenylosuccinic acid lyase, it was necessary to have on hand a relatively pure preparation of the lyase. The customary source of this enzyme is Baker's yeast, but the published purification techniques are tedious. We therefore explored the use of preparative isoelectric focusing in slurries of Sephadex. Following autolysis of 1 kg of Baker's yeast, a high-speed supernatant was prepared and dialyzed against 0.001 M Tris HCl, pH 7.8, containing 10 mM 2-mercaptoethanol. The low-salt extract was mixed with 8 g of Sephadex G-75, superfine, 5 ml of Ampholines (pH 3.5-10) and 50 μ l of 2-mercaptoethanol. The slurry was flattened by spatula into a smooth sheet and focused directly without the ordinary antecedent drying step. Many active isozymes were observed, but the major species focused at pI's of 4.4 and 5.2. The latter of these was purified 30 fold in this single first step and was free of adenylosuccinic acid synthetase. Pursuant to its purification, this fraction was tested for its ability to decompose the adduct of L-Alanosine with AICOR; treatment of the adduct with alkaline phosphatase from E. coli was also carried out. Both enzymes altered the chromatographic behavior of "L-Alanosyl Aminoimidazolecarboxylic Acid Ribotide" and abrogated its inhibition of adenylosuccinate synthetase. Analogous studies with adenylosuccinate lyase and alkaline prosphatase of mammalian origin are planned.

I. New Methodology for the Measurement of the Enzyme that Conjugates L-Aspartic Acid (and L-Alanosine) with 5'-Phosphoribosyl 5-Aminoimidazole 4-Carboxylic Acid (AICOR) Kinosynthase. The kinosynthase in question uses ATP.MgCl$_2$ as energy source; ADP.MgCl$_2$ and Pi are the products. Over twenty years ago, J. Buchanan demonstrated that the reaction was reversible, i.e., that L-aspartic acid could be generated from 5'-phosphoribosyl-4(N-[^{14}C] succinocarboxamide)-5-aminoimidazole in the presence of phosphate and ADP. He also showed that arsenate accelerated such reversibility. We have capitalized on these observations in the development of a new analytical approach to this kinosynthase: 5'-phosphoribosyl-4-(N-[^{14}C]succinocarboxamide)-5-aminoimidazole (specific radioactivity: 19.5 μCi/ μmole), was prepared from AICOR and L-[4-^{14}C]aspartic acid in the presence of ATP.MgCl$_2$ using kinosynthase purified from chicken liver; precursors were separated from products on a column of Dowex 1 x 8, formate, using a linear gradient of 1 L each of water and 4.0 M formic acid. The strongly acidic new nucleotide, which eluted at 3.0 M acid, was lyophilized and reconstituted with water to a concentration of 12.5μCi/ml. Five μ l of this solution was mixed with 0.01 M ADP.MgCl$_2$ in 0.01 M sodium arsenate buffer, pH 8.0. The reaction was initiated by the addition of organ extract. After 15 minutes at 37^o, 50 μ l of a decarboxylation reagent (which quantitatively detaches the 4th carbon of L-aspartic acid) is added to the reagents, at the same time as a droplet of 40% KOH is deposited on the underside of the lid of the vessel. After 3 further hours at 37°, the radioactivity trapped on the lid is quantified by scintillation spectrometry. The assay described is sensitive enough to detect the formation of as little as 100 pmoles of product, and is notably facile. Use of it has been made to measure the specific activity of the kinosynthase in homogenates of the principal viscera of Mus musculus. In this survey, eyes contained the enzyme at highest specific activity (∿25 nanomoles/g/ hr) followed by kidney (∿ 18 nanomoles/g/hr) pancreas (∿ 17 nanomoles/g/hr) and brain (∿14 nanomoles/g/hr). Small intestine exhibited the lowest activity (∿2.5 nanomoles/g/hr). It should be pointed out that all of these activities are at least 10-50 fold lower than that present in chicken liver. Use also has been made of the assay to assess the specific activity of the kinosynthase in tumors resistant to L-Alanosine and their sensitive counterparts. Three systems were available for this purpose: 1) a line of neuroblastoma cells in culture able to grow in the presence of concentrations of L-Alanosine 130 times those killing 50% of the sensitive counterparts: (670 μM vs 5 μM); lines of L1210 and P388 rendered resistant to doses of L-alanosine (40 mg/kg) capable of producing increased life span of 50-80% in mice bearing the native, sensitive lines. These resistant lines were developed and kindly provided by Dr. R. Johnson of ADL. On assay, the specific activity of the kinosynthase was not significantly different in any of these lines; thus, it does not appear to be a determinant of sensitivity to Alanosine. Conversely, the specific activity of adenylosuccinate lyase, (the enzyme thought to catalyze the decomposition of the adduct of L-alanosine with AICOR) was elevated 4 and 8 times in the sensitive L1210 and P388 lines respectively. This paradoxical result merits further examination.

J. Influence of L-Alanosine on IMP Pools. Other things being equal, L-alanosine, by virtue of its inhibition of adenylosuccinate synthetase ought to produce an elevation of the concentration of IMP. In fact, this metabolic perturbation does occur when cultured Novikoff hepatoma cells are exposed to the drug in vitro. Using a sensitive double-beam spectrophotometric assay, we were unable to demonstrate an analogous elevation in extracts of tumor and liver 1 hour after the administration of L-alanosine at a dose of 500 mg/kg. This result was perplexing. Accordingly, the experiment was repeated and expanded to include additional time-points. High Pressure Liquid Chromatography was used to resolve IMP from other nucleoside monophosphates. At 1 and 3 hours after a dose of 500 mg/kg, there was no significant change in the hepatic concentration of inosinic acid; at 5 hours, a 33% reduction in the pool size of this nucleotide was observed in liver. This result is the opposite in direction to what would be anticipated on metabolic grounds but may be explained by invoking a compensatory increase in the activity of IMP-dehydrogenase, an enzyme capable of siphoning-off the excess nucleotide.

K. Potent Inhibitors of L-Asparagine Synthetase. During the course of our continuing search for inhibitors of L-asparagine synthetase, two important agents were uncovered.

1. Leucine chloromethyl ketone, HCl. Chloromethyl ketones are widely used as active-site directed, irreversible inhibitors of a broad spectrum of enzymes. Leucine chloromethyl ketone is an effective inhibitor of the transport of L-leucine across cell membranes. This compound was examined as an inhibitor of L-asparagine synthetase and was found to be a potent inhibitor of utilization of L-glutamine and ammonia by L-asparagine synthetase from L5178Y/AR and from normal mouse pancrease (Ki=0.1 mM). In vitro this compound appears to be capable of reacting with one or more sulfhydryl functions at the active site of the enzyme: thus, both L-glutamine and dithiothreitol protect against inhibition by it. Inasmuch as this agent failed to inhibit L-asparagine synthetase in vivo or in a suspension of isolated L5178Y/AR cells, it is the strong presumption that its entry into the intracellular domain has been precluded by interaction with the transport machinery. If, however, it should prove to have chemotherapeutic activity, it will be a first or early representative of a new class of oncolytics: transport inhibitors.

2. 3-(Phosphonoacetylamido-L-alanine)(PA_2LA). This phosphonoamino-acid was synthesized as a transition state inhibitor of L-glutamine synthetase by Dr. F. Wedler. Because it is possible that the β-carboxyl of L-aspartic acid is transitorily esterified with a phosphoryl group of ATP or AMP in the course of the reaction catalyzed by L-asparagine synthetase, and for structural reasons, we examined PA_2LA as an inhibitor of this latter enzyme from pancreas and L5178Y/AR. Both enzymes were inhibited approximately 50% at an inhibitor concentration of 500 μM. Mechanistic studies of this effect are underway.

L. L-Asparagine Synthetase as a Predictor of Responsiveness of Acute Lymphocytic Leukemia (ALL) to Enzyme-Chemotherapy. Most cases of ALL fall into one of three classes: null-cell, T-cell and B-cell. The response of these cell types to chemotherapy (with L-asparaginase and other modalities) is radically different. Null and T-cells are good responders to L-asparaginase and to L-asparagine deprivation in vitro, while B-cells are poor responders. The present study was directed at quantitating the levels of L-asparagine synthetase in these types of leukemic cells grown in tissue culture: activities of 0.71 ± 0.19, 1.0 ± 0.6 and 1.1 ± 0.6 nmoles of L-asparagine synthesized/mg protein/hr were determined in the null-cell types (2 lines), T-cell type (4 lines) and B-cell type (5 lines), respectively. It is concluded that the specific activity of L-asparagine synthetase in cultured lymphoblasts is not a function of immunologic types.

M. Studies with L-Asparagine. Free L-aspartic acid in crystalline preparations of L-asparaginase from Escherichia coli. Each month the National Cancer Institute dispenses approximately 5000 vials of L-asparaginase from E. coli for the treatment of ALL. This drug is also one of the comparatively few oncolytics with an NDA. Serendipitously, in the course of amino acid analysis of this molecule, we observed that between three and four moles of free L-aspartic acid were affiliated with every mole of L-asparaginase. As judged by equilibrium dialysis, four binding sites for this amino acid exist on the enzyme, which is known to be a tetramer. The presence of four binding sites was also confirmed by protein fluorescence spectrophotometry. For this study, 10 nmoles of L-asparaginase was dissolved in 2 ml of 0.05 M Tris-buffer, pH 8.5; then aliquots of 7.5 nmoles of L-aspartic acid were added and fluorescence measurements (excitation at 290 nm and emission at 324 nm) were carried out at 20^o. Binding constants were calculated using percent change in fluorescence. It is noteworthy that the free L-aspartic acid can be removed by transamination or decarboxylation without detriment to the specific activity of the enzyme; however, L-aspartic acid-free L-asparaginase, so prepared, exhibits only two binding sites for its product. When the enzyme was exposed to various group-modifying reagents, it was found that those treatments which destroyed catalytic activity also diminished the binding of L-aspartic acid. It is concluded that the affiliation of free L-aspartic acid with L-asparaginase is non-covalent but pertenacious and that it introduces a modest error in the determination of the amino acid composition of this important therapeutic enzyme.

N. Studies with Fluorouracil. Development of an In Vitro Predictive Assay of Responsiveness. We have developed a simple *in vitro* predictive assay for tumor sensitivity to 5-FU. The two most important metabolites of 5-FU are: 1) 5-fluorodeoxyuridine monophosphate (5-FdUMP), an exceedingly potent inhibitor of thymidylate synthetase; and 2), 5-fluorouridine triphosphate which is known to be used for, and also to interrupt RNA synthesis. The *in vitro* assay system we have developed requires 50 mg of tumor which is homogenized in a buffer containing Tris, EDTA and ditheothritol and centrifuged at 12,000 g. A 10 µl aliquot of $[^{14}C]$5-FU is added to 10 µl of a buffer mix containing a combination of sugars, ribose-1-phosphate, 2 deoxyribose-1-phosphate and phosphoribosyl pyrophosphate at 0.03 M, pyruvate kinase, myokinase, ATP and 0.03 M NaF at pH 8.0. The reaction is initiated with the addition of a 10 µl aliqout of cytosol and the mixture is incubated at 37^o for 30 minutes then heated at 95^o for 2 minutes. Following centrifugation, the supernatant is assayed by five methods: 1) paper chromatography and 2) ion exchange chromatography, both of which serve to quantitate the total phosphorylated metabolites of 5 FU formed under the conditions of incubation; 3) high resolution column chromatography, which allows an analysis of anabolism of the base to mono, di and triphosphates (without distinguishing ribotides from deoxyribotides); and 4) enzymatic measurements of FdUMP. These measurements were made by published techniques modified in such a way that micro-distillation was the last step; (cf. adenylosuccinate lyase); and, 5) analysis of alkaline phosphatase activity. In a series of murine tumors, selected to range from the most sensitive to the least sensitive to 5-FU, total phosphorylation was found to be proportional to the sensitivity or resistance to 5-FU, *in vivo*. Phosphorylation of 5-FU, *in vitro*, by a series of human adenocarcinomas was in general markedly lower than what was seen in murine tumors. Nevertheless, the extracts of many human breast adenocarcinomas generated significantly greater amounts of FdUMP from 5-FU than their murine counterparts. Analysis of tumor alkaline phosphatase in 5-FU sensitive and resistant P388, demonstrated a ten fold increase in the concentration of the enzyme in the resistant as compared with the sensitive line.

It is concluded that the rate of total phosphorylation of 5-FU by murine tumors in vitro can provide an index of their responsiveness to the drug. Measurements of the rate of generation of FdUMP also suggest there are species specificities operative. Measurement of the higher anabolites, such as di and triphosphates in extracts of tumors provides insight into their deficiency in specific kinases. Such measurements coupled with estimations of alkaline phosphatase also permit an assessment in vitro of the ability of tumors to decompose the active anabolites. In the case of at least one murine tumor resistant to FU, (P388/FU) high phosphatase appears to explain the refractory state.

Significance to Biomedical Research and the Program of the Institute. Ideally, the Biochemistry Section uncovers the biochemical mechanisms underlying the toxicity of new oncolytic drugs and suggests rational antidotes for this toxicity. The stress is on enzymology, inasmuch as most drugs operate by interacting with enzymes. Practically speaking, this unit cooperates with the Laboratory of Toxicology as a whole, and with the Division of Cancer Treatment in ensuring that new chemotherapeutic modalities can be used safely in man.

Proposed Course. It is proposed to pursue studies on new oncolytic agents examining their toxicity at the level of enzymes, cells and in the whole animal.

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3. Moore, L., Landon, E. J., and Cooney, D. A.: Inhibition of the Cardiac Mitochrondrial Calcium Pump by Adriamycin in vitro. Biochem. Med. 18: 131-138, 1977.

4. Cooney, D. A., Milman, H. A., Cable, R. G., Dion, R. L., and Bono, V. H., Jr.: Maleimide-Biochemical, Pharmacologic and Toxicologic Studies, Interaction with L-Asparagine Metabolism. Biochem. Pharm. 27: 151-166, 1978.

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6. Cooney, D. A., Karlowicz, M. G., Cubillan, J., Roettger, M., and Jayaram, H. N.: An Indirect Radiometric Technique for Measuring N-Phosphonacetyl-L-Aspartic Acid (PALA) in Tissues. *Cancer Treat. Rept.* (in press), 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06110-08 LT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Pathology of Drug Induced Alterations in Animals and Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS: 1.3	PROFESSIONAL: 1	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective of this project is to define the morphologic effects of drugs in animals and to carry these observations to the biochemical/morphologic level; these findings are then compared with those reported in man. A secondary objective is to define animal models of human disease.

The specific studies involve the following:

1. The clincopathologic effects of Adriamycin in rabbits, rats and mice.
2. The effects of CoQ10, Vitamin E and similar substances on the Adriamycin-induced cardiotoxicity in the rabbit model.
3. The evaluation of the rat as a model for testing the nephrotoxicity of platinum containing antineoplastic agents.

Project Description:

Objectives:

1. To define the clinicopathologic effects of adriamycin (ADR) and to extend such morphologic observations to the molecular/biochemical level in rabbits, mice and rats and characterize an animal model for:
    a. analogue testing
    b. antidotal testing
    c. antitumor and cardiotoxicity testing in parallel
2. To test the following ADR analogues and antidotal procedures in the rabbit model to determine their effects on the development of the cardiomyopathy.
    a. Quelamycin
    b. Coenzyme Q_{10}
    c. Vitamin E
3. To evaluate the rat as a model for testing the nephrotoxicity of platinum containing antineoplastic agents.

Major Findings:

A. The Clinicopathologic Effects of ADR in Rabbits, Rats, and Mice. Recent reports of the toxic effects of ADR in animals and humans indicate that the tissues of the following eight organ systems are adversely affected: hematopoietic, lymphoreticular, digestive, reproductive, cardiovascular, urinary, skeletal and integumentary. Myelosuppression, digestive disturbances, stomatitis and alopecia are the most frequently occurring adverse effects in patients receiving ADR. It has been well accepted that the cardiotoxicity of ADR is the major total dose-limiting toxic manifestation in patients. A number of animal models have been characterized to various degrees as indicators of ADR-induced cardiotoxicity. An animal model would be valuable in testing analogues of ADR for cardiotoxicity and for testing antidotal procedures. To date, only the rabbit and monkey have been reported to develop a dose-related cardiomyopathy with congestive heart failure. Smaller laboratory animals (i.e., mouse and rat) offer distinct advantages as models with respect to size, temperament, handling, care and housing required for testing large numbers of drugs. Therefore, the current investigations were designed to determine if ADR would induce a significant cardiotoxic syndrome in the rat. ADR was administered to rats at doses of 1-2 mg/kg per week for 10-14 weeks. The majority of ADR-treated rats developed cardiomyopathy from 3 to 23 weeks after the last injection. Forty to seventy percent of the rats with cardiomyopathy had gross evidence of congestive heart failure (pleural effusions, ascites, hepatomegaly and cardiomegaly). Histologic myocardial changes consisted of myocyte vacuolation and degeneration, interstitial edema and mild fibroplasia. In addition, damage to atrial ganglion cells was evident in several rats. Ultrastructural alterations involved sarcoplasmic disruption with distentions of subcellular organelles and loss of myofilaments. The myocardial lesion is similar to that described in the rabbit, although it is less severe. Extra-cardiotoxic effects of ADR in the rat (nephrotoxicity, myelosuppression, enteropathy and arrested osteogenesis) were minor and observed most frequently at high cumulative doses.

The results of the present study suggest that the rat model is an accurate, reproducible, cost effective system for large scale cardiotoxicity testing of analogues of ADR and daunorubicin (DNR).

B. The Effect of CoQ_{10} on Adriamycin-Induced Cardiotoxicity in the Rabbit Model. The present studies were designed to determine the ability of CoQ_{10} (C) to counteract the toxic effects of ADR, specifically the cardiomyopathy (CMY). Forty-four male NZW rabbits were grouped accordingly: ADR-C (12), ADR-vehicle (12), ADR-saline (12) and C-saline (8). C was administered intravenously five mornings/wk at a dose of 1 mg/kg commencing five days before ADR administration. ADR was administered intravenously at a dose of 0.8 mg/kg (8.8 mg/M^2) three consecutive afternoons/wk. Vehicle and control rabbits received similar volumes intravenously at the same schedule. ADR-C rabbits had a mean survival of 81 days, a mean total ADR dose of 305 mg/M^2 and an incidence of CMY of 83%. These findings were not significantly different from those of the ADR-vehicle group (89 days, 336 mg/M^2, 83% incidence) or the ADR-saline group (90 days, 340 mg/M^2, 75% incidence). Semiquantitative estimation of the CMY at the light microscopic level revealed a slight reduction in the severity of the lesion in the ADR-C group compared to the other ADR treatment groups. These results indicate that C does not significantly counteract either the general toxic effects of ADR or the incidence of CMY.

C. Evaluation of Quelamycin (Triferric ADR NSC-267,703) for Cardiotoxic Activity in the Rabbit Model. Previous reports from Professor Mario Gosalvez's laboratory suggest that Quelamycin is non-cardiotoxic based on inhibitory activity against mammalian cardiac Na-K and shark rectal gland ATPase. Four groups of six rabbits each were given Quelamycin (supplied by Montedison Farmaceutica, Barcelona, Spain) I.V. twice/week at doses 1x, 2x, 3x, and 4x the cardiotoxic dose of ADR (1.2 mg/kg). Rabbits in the two higher dose groups died from toxicity at 9-23 days after initiation of treatment. Histopathologic evaluation of selected tissues revealed severe bone marrow hypoplasia and no evidence of myocardial lesions. Rabbits in the 2x group died from toxicity over a period of 14-81 days after start of treatment, at a maximum total dose of Quelamycin of 634 mg/M^2. Two of the rabbits had gross evidence of congestive heart failure at the time of necropsy. The other four rabbits exhibited diffuse petechial and ecchymotic hemorrhages on serosal surfaces of abdominal and thoracic viscera. These findings were interpreted as indications of severe myelosuppression. Five rabbits survived total doses of Quelamycin of 650 mg/M^2. These preliminary findings indicate that: (a) Quelamycin is cardiotoxic in the rabbit model; and (b), Quelamycin is approximately one-third as cardiotoxic as ADR. This analog may not offer any advantage over ADR since the oncolytic activity of Quelamycin is 1/3 that of ADR in experimental tumor systems.

D. Evaluation of the Effects of Vitamin E on the Cardiotoxic Activity of ADR in the Rat and Rabbit. Acute Studies in the Rat - Five groups of 10 rats each were designated as follows: (1) ADR (I.P.) + Vit. E. (I.P.); (2) ADR (I.P.) + Saline (I.P.); (3) Vit. E. (I.P.) + Saline (I.P.); (4) ADR (I.P.) + Vit. E. (S.Q); and (5), Vit. E. (S.Q.) + Saline (I.P.). Vitamin E (Sigma No. T-3634) was injected at a dose of 3,000 I.U./kg 24 hours prior to treatment with ADR (I.P. 20 mg/kg). The 14 day survivors for the five groups were 30%, 20%, 100%, 80% and 100% respectively. Selected tissues were fixed and processed for histopathologic evaluation. No lesions were observed in the myocardia of any of the rats. Rats receiving ADR had severe peritonitis, enteritis and lymphocytic hypoplasia of the thymus and spleen. These preliminary findings indicate that I.P. Vit. E. does not protect significantly against the lethality of I.P. ADR. Current studies are in progress to verify the apparent protection against I.P. ADR-induced lethality with prior S.Q. administration of Vit. E.

Acute Studies in the Rabbit - Six groups of 4 to 6 rabbits were designated as follows: (1) Vit. E. (I.P.) + ADR (I.P.); (2) ADR (I.P.) + Saline (I.P.); (3) Vit. E. (I.P.) + Saline (I.P.); (4) Vit. E. (S.Q.) + ADR (I.V.); (5) ADR (I.V.) + Saline (S.Q.); and (6) Vit. E. (S.Q.) + Saline (I.V.). Vitamin E (Sigma No. T-3634) was injected at a dose of 3,000 I.U. 24 hours prior to treatment with ADR (8 mg/kg). The 14 day survivors of the treatment groups were 0%, 75%, 100%, 0%, 0% and 100%, respectively. Two additional control rabbits received 3,000 I.U. of Vitamin E. I.P. and were sacrificed 24 hours post-injection. Samples of parietal and visceral peritoneum were collected and processed for histopathologic examination. Microscopic evaluation revealed a moderate to marked granulomatous peritonitis with a predominance of mononuclear cells and neocapillary formation. Rabbits receiving ADR I.V. had severe bone marrow hypoplasia with disseminated petechial and ecchymotic hemorrhages irregardless of whether they had received Vitamin E. No lesions were evident histopathologically in any of the hearts from the test rabbits. These preliminary findings suggest that: (a) Vitamin E does not protect against the acute lethality of ADR in rabbits; (b) the rabbit peritoneum is highly sensitive to injury; (c) the preparation of Vitamin E used is toxic to rabbit peritoneal surfaces; and (d), I.P. ADR inflicts its damage locally (peritonitis) with death occurring before any characteristic manifestations of systemic toxicity (i.e. myelosuppression) are evident.

E. Evaluation of the Rat as a Model for Testing the Nephrotoxicity of Platinum Containing Antineoplastic Agents. Nephrotoxicity is the major dose limiting toxicity in patients receiving Cis-diamminedichloroplatinum II (NSC-119,875). Six agents containing platinum (NSC-119,875, 250427, 239851, 180512, 224964, and chloroplatinic acid) were administered parenterally to 500 male F344 rats, 6 weeks of age, at various dose levels from LD_{10}-LD_{100}. This study showed that all six platinum-containing drugs were nephrotoxic by I.P. and/or I.V. administration. Two drugs with minimal renal toxicity by I.P. administration were highly toxic to the kidney after I.V. injection. The nephrotoxicity of chloroplatinic acid verified the potent toxicity of platinum-containing compounds to renal tubules. The most severe renal toxins (chloroplatinic acid, NSC-239851, platinum uracil blue, and NSC-250427) resulted in uremia and renal failure as the cause of death and produced necrotizing lesions of the proximal tubules throughout the entire renal cortex. The less nephrotoxic drugs [cis-DDP(II) and NSC-224964] and lower doses of the more nephrotoxic drugs induced lesions in proximal tubules of the medullary outer strip. The results of these studies suggest that platinum compounds should be considered as potent renal toxins along with other heavy metals.

All platinum-containing agents induced degenerative and regenerative lesions in the proximal tubules associated with changes in BUN and creatinine levels. The results of our work and that of others indicate that the Fischer rat provides a sensitive strain for testing potentially nephrotoxic drugs since the lesions are the same as found in man.

F. Evaluation of the Ability of Furosemide to Modify the Renal Toxicity Induced by Platinum Containing Anticancer Agents. Cis-Dichlorodiammine-platinum (II)(DDP) produces toxic effects in the hematopoietic, auditory, gastrointestinal, and urinary systems. Nephrotoxicity in man and rats appears to be a dose-limiting toxicity. Diuretics administered to patients treated with this platinum drug appeared to modify its nephrotoxicity. The present study was designed to further investigate in rats the apparent amelioration of this important toxic effect.

As early as 1-2 days after administration of a single dose of DDP in male F344 rats, the blood urea nitrogen (BUN) levels increased and reached a peak 4-5 days later, returning to near normal by Day 14. The elevated BUN levels were correlated with degenerative, necrotizing, and regenerative renal tubular lesions in the pars recta of the proximal tubules. Elevated BUN levels on Day 3 were shown to be dose-related and also associated with the degree of renal damage. Although furosemide decreased urinary excretion of DDP by maintaining high plasma and renal levels of platinum at least during the first 24 hours, the diuretic attenuated the chronic renal failure induced by weekly administration of DDP.

G. Computer-Assisted Evaluation of Adverse Tissue Effects of Antineoplastic Agents in Cancer Patients as Revealed by Postmortem Examinations. The results of 600 autopsies of patients treated for neoplasia by chemotherapy, irradiation, surgery or combinations of 2 or all of the modalities have been entered into computer storage and programmed for ease of retrieval. Of these 600 patients, 324 (54%) are males and 276 (46%) are females. They ranged in age from 2 to 90 years with nearly half being between the ages of 31 and 60. Thirty-seven were 10 years or younger and 28 were above the age of 70. Most patients had either leukemia or lymphoma. One hundred twenty (20%) had acute leukemia (ALL or AML) and 72 (12%) had chronic leukemia (CML or CLL). Eighty three (14%) had non-Hodgkin's lymphoma and 59 (10%) had Hodgkin's lymphoma. Sixty-eight had adenocarcinoma, 47 squamous cell carcinoma, 36 melanoma and 30 various sarcomas. The remainder were divided into rarer neoplasias such as germ cell, brain and plasma cell tumors. Of the 600 autopsies, 464 were performed in the NCI laboratory and 136 were reviews of submitted slides from autopsies done on NCI patients dying elsewhere. Five hundred forty-five (91%) were treated with antineoplastic agents, 306 (51%) with irradiation and 34 (6%) by immunotherapeutic regimens such as intracutaneous BCG or derivatives thereof.

Information concerning at least 46 drugs was stored. These were mostly antineoplastic agents but also included some others such as selected antibiotics, allopurinol and INH. Twenty-three drugs were given to at least 25 patients. The 6 drugs used most frequently and listed in order of frequency were: corticosteroids (326, 54%); vincristine (322, 53%); cyclophosphamide (247, 41%); methotrexate (196, 33%); ara-C (156, 26%); and 6 MP (121, 20%). Charts have been made which list each patient, diagnosis, and the drugs given.

Using the coder in the Systemized Nomenclature of Pathology, the principal morphologic changes were: inflammation (409, 68%); fibrosis (367, 61%); atrophy (357, 60%); necrosis (279, 47%); deposition of materials such as fat, amyloid and calcium (246, 41%), and acute inflammation (242, 40%).

H. Significance to Biomedical Research and the Program of the Institute. The need for a better understanding of the pathogenesis of anticancer drug-induced lesions in experimental and clinical studies cannot be overemphasized. The elucidation of the biochemical/molecular mechanisms of ADR-induced cardiotoxicity may lead to establishment of a therapeutic or prophylactic procedure(s) to negate the cardiac failure syndrome.

Detailed characterization of appropriate experimental animal models is of paramount importance in investigative studies. The characterization of animal models of organ specific toxicity for evaluation of analogues of parent drugs is an important aspect of toxicologic studies. The results of present studies indicate that the rat is a good model for ADR-induced (chronic) cardiotoxicity and for platinum-induced renal toxicity.

I. Proposed Course. Intramural research activities will be continued and expanded under the specific objectives: a) To characterize the cardiomyopathic effects of ADR by extending histopathologic-morphometric analysis to the molecular level; b) To define modalities and/or analogues to alleviate the cardiomyopathic effects of the anthracycline class of agents; and c), To develop and establish techniques in the area of quantitative morphometric analysis of tissues containing drug-induced lesions.

Publications:

1. Ward, J. M., Grabin, M. E., LeRoy, A. F., and Young, D. M.: Modification of the renal toxicity of cis-diamminedichloroplatinum (II) (NSC-119,875) with furosemide in male F344 rats. Cancer Treat. Rept. 61: 375-379, 1977.

2. Mettler, F. P., Young, D. M., and Ward, J. M.: Adriamycin-induced cardiotoxicity in rats: an animal model for analogue testing. Cancer Res. 37: 2705-2713, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06122-06 LT

PERIOD COVERED
October 1, 1977 - September 30, 1978

TITLE OF PROJECT (80 characters or less)

Drug Interactions and Other Factors Influencing the Physiological Disposition and Toxicity of Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8	6	2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In recent years, it has become common practice, particularly in clinical oncology to simultaneously administer several drugs to patients for therapeutic purposes. A vast literature, both experimental and clinical, has shown that the presence of one drug may markedly influence the intensity, duration, and pharmacologic effects of another drug. Such drug interactions may have a variety of causes: they may result from alterations in drug absorption, in the binding of drugs to plasma proteins, which may directly or indirectly influence drug distribution, drug metabolism, or drug clearance via the bile or urine. Many drugs and other foreign chemical compounds known collectively as xenobiotics - are inactivated by enzymes present in liver microsomes, primarily by oxidation and conjugation. On the other hand some drugs and oncolytic agents are activated by these enzymes. Our major efforts are directed at understanding how drugs interact with each other and with biological organisms so that human drug therapy can be based on more rational grounds.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The interaction between a drug and tissue receptors which ultimately produces a pharmacologic or toxicologic response is the product of a large number of attenuating factors. For example, the distribution of drugs within the body depends upon a balance between relative organ perfusion, the affinity of the drug for binding sites within different organs, the extent of binding of the drug to plasma proteins, and the rate of its excretion into the urine and bile. Moreover, the metabolic clearance of many drugs from the body depends upon their relative rates of biotransformation to products which are more polar and more rapidly excreted. Thus, absorption, distribution, binding, metabolism and excretion tend to determine the steady-state plasma drug concentration under specified conditions and thus determine the pharmacologic or toxicologic response. Since alterations in these factors are known to either diminish or intensify biologic responses, the need to assess these parameters quantitatively is of obvious therapeutic significance. Studies have been continued in the Drug Interactions Section to investigate and appraise these parameters in a variety of animal species regarding drugs and other xenobiotics in general, and oncolytic agents in particular. A large body of evidence has linked drug metabolism to drug disposition and the duration of drug action. Only recently have data accumulated which emphasize the important role of drug metabolism in drug-induced toxicity. Recent findings have demonstrated that many relatively inactive foreign chemical compounds can be enzymatically activated *in vivo* to what might be termed *proximate toxicants*.

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

A. The Effect of Glucocorticoids on the Morphologic and Biochemical Development of Mixed Function Oxidase Activity in Prenatal Rabbit Lung. A number of published reports have indicated that treatment of prenatal rabbits with natural (cortisol) or synthetic (9 α-fluoroprednisolone) adrenal glucocorticoids accelerates or causes premature:

1. Morphologic maturation of lung as determined by light microscopy.

2. Preferential maturation of pulmonary type II cells, particularly the cytoplasmic structures as revealed by electron microscopy.

3. Increases in pulmonary surfactant content.

Since it is well documented that hepatic cell maturation is generally associated with increased mixed function oxidase (MFO) activity, it seemed plausible that glucocorticoid mediated accelerated maturation of pulmonary type II cells might result in increased pulmonary MFO activity. Rabbit fetuses were injected *in utero* with cortisol on day 25 of gestation and sacrificed 48 hours later. Histologic examination of the lungs from cortisol-treated fetuses confirmed accelerated lung maturation by comparison with saline-injected controls. However, no significant differences were found between cortisol and saline-treated fetal rabbits in hepatic or pulmonary NADPH cytochrome c reductase, native or 'activited' UDP-glucuronyl-transferase, N-acetyl-transferase or glutathione S-aryl-transferase. Cytochrome P-450, aminopyrine N-demethylase and biphenyl-4-hydroxylase could not be detected in fetal rabbit lung or liver in either group. Thus, although it is clear that the administration of cortisol to fetal rabbits accelerated lung maturation, no corresponding increase in pulmonary drug metabolism could be demonstrated.

B. The Effect of Chlorpromazine on Paraquat Toxicity and the Uptake of Paraquat by Rat Lung. Ingestion or injection of the herbicide paraquat (PQ) in experimental animals or humans results in acute pneumonitis and pulmonary edema which usually progresses inexorably to pulmonary fibrosis and death from pulmonary insufficiency. As yet, no effective therapy for PQ poisoning is known. As part of an extensive study investigating the uptake of PQ into rat lung slices, several drugs were examined for their ability to inhibit PQ accumulation and enhance efflux of previously accumulated PQ. Several of the compounds that were tested had such activities but chlorpromazine (CPZ) appeared to be the most potent and warranted further studies. The inhibition of PQ uptake by CPZ was dependent upon the concentration of the inhibitor in a hyperbolic manner. In the absence of CPZ, PQ (10 μM) uptake into lung slices was linear with time for up to two hours. In the presence of 100 or 300 μM CPZ, uptake at a reduced rate was linear for only about one hour and thereafter decreased or completely ceased. In another series of experiments, the efflux of PQ from rat lung slices into fresh medium was investigated. A number of basic drugs either inhibited efflux or were without effect but CPZ enhanced efflux of PQ from lung slices, in a concentration - dependent manner. These effects of CPZ on PQ uptake into and efflux from lung slices raised the intriguing possibility that a similar interaction may also take place *in vivo* and, if so, might protect animals against the pulmonary toxicity of PQ. To test this, rats were injected with CPZ (s.c.) and then with PQ (i.v.) 30 minutes later. Daily counts were made of the number of deaths. To our surprise, CPZ did not produce the expected protective effect but instead potentiated the toxicity of PQ. This was found to be due to a reduced renal clearance and increased lung concentration of the herbicide in presence of CPZ. When saline (p.o.) was administered with CPZ and PQ, the renal clearance was increased, the lung PQ content was normalized and PQ-potentiation was markedly reduced. These results indicated that although CPZ inhibited PQ uptake and enhanced PQ efflux in rat lung slices, it did not protect against PQ poisoning *in vivo*.

C. The Effect of Vitamin A Deficiency on Drug Metabolism. Evidence has accumulated suggesting that vitamin A deficiency renders animals more susceptible to certain chemical carcinogens. Although hepatic drug metabolism in vitamin A deficient animals has been studied, the results from various laboratories have not been consistent. This may be due to differences in length of time that the animals were maintained on the deficient diet and on their severity of the deficiency. Our study, therefore, was initiated to examine the MFO system at varying stages and severities of deficiency. Three, 4, 5 and 6 weeks after placing rats on a vitamin A deficient diet, when the hepatic levels of vitamin A were undetectable (<1 μg/g), cytochrome P-450, NADPH cytochrome c reductase, aniline hydroxylase, biphenyl hydroxylase, native and 'activated' UDP-glucuronyl-transferase, N-acetyltransferase and N-demethylase activities remained unaltered as compared to the controls. However, glutathione S-aryl transferase activity was unchanged at 3 weeks but increased approximately two-fold at 4, 5 and 6 weeks of deficiency. Experiments are underway to examine *in vivo* and in isolated organs the effect of vitamin A deficiency on drug metabolism involving glutathione conjugation.

D. Effects of Ascorbic Acid Supplementation on Hepatic Drug Metabolism in Guinea Pigs. Deficiency of ascorbic acid has been repeatedly shown to impair hepatic microsomal drug metabolism and to prolong the action of certain drugs. Conversely, recent studies have suggested that ascorbic acid supplementation might increase drug metabolism to above control levels in a roughly dose-dependent fashion, but because of the design of these experiments, the results are difficult to interpret. The purpose of the present study was to determine whether maintenance of animals on diets which differed only in their content of ascorbate would affect levels of hepatic drug metabolism. Guinea pigs were maintained on four diets which contained 0.3, 1.5, 3.5, and 7.0 mg ascorbic acid per g of diet. The lowest diet contained sufficient ascorbic acid to prevent scurvy, while the two diets highest in ascorbic acid content produced maximum hepatic ascorbate concentrations after eight weeks. Several hepatic enzymes were measured after 1, 2, 3, 4 and 8 weeks. There were no significant differences among the four groups in the following parameters of microsomal mixed function oxidation: cytochrome P-450 content, NADPH cytochrome c reductase, and aminopyrine N-demethylase. Also, there were no differences in native and nucleotide-activated UDP-glucuronyl transferase activities. However, in the post-microsomal supernatant, overall glutathione S-aryl transferase activity was significantly lower in the group with the lowest supplementation of ascorbic acid, as compared to the three higher groups. Kinetic parameters of aminopyrine N-demethylation were studied in animals maintained for 21 days on a diet deficient in ascorbic acid (<0.1 mg ascorbic acid/g), an intermediate control diet (1.5 mg ascorbic acid/g) and a high-dose ascorbic acid diet (7.0 mg ascorbic acid/g). Curvilinear Lineweaver-Burk plots were obtained in all three groups, and apparent K_m and V_{max} calculated separately for both low and high ranges of substrate concentrations. Apparent K_m's did not differ significantly among the three groups. Microsomes from ascorbic acid deficient animals had V_{max} values lower than the control and high-dose groups. There was no difference in kinetic parameters between microsomes from animals receiving control and high-dose ascorbic acid diets.

E. Studies on the Possible Role of Protein Synthesis in Cell Injury by Free Radical Pathogens. Several theories have been advanced in attempts to explain the subcellular processes terminating in chemically-induced cell death but none is entirely satisfactory. Alterations once considered as critical to explain cell necrosis were later found either insufficient to account for the effects or not to be general features in the action of toxic compounds. It may very well be that no unifying mechanism of cellular necrosis is operative and that no single alteration in a given organelle may by itself provide a common pattern for necrosis produced by different toxic compounds. In recent years, however, it has become apparent that protein synthesis may play an active role in the production of cellular damage. This possibility arose from experiments in which inhibitors of protein synthesis were found to partially prevent chemically-induced cellular necrosis as well as some other manifestations of the tissue damage caused by several cytotoxic agents in the rat liver and pancreas. The question arose as to whether the protective effect of these inhibitors was related to their activity as inhibitors of protein biosynthesis, per se, or to some non-specific, indirect effect. One of the inhibitors of protein synthesis frequently employed in such studies was cycloheximide (NSC-185). In the present work we sought to discover if cycloheximide administration had any effect on parameters known to be modified during CCl_4-induced liver necrosis. Cycloheximide pretreatment (1 mg/kg, i.p.) slightly attenuated the hepatic necrosis 24 hr after CCl_4 administration and this attenuation was more obvious at 72 hr. A larger dose of cycloheximide (2 mg/kg) evoked a marked antidotal effect even at 24 hr.

Prior treatment of the rats with cycloheximide (1 mg/kg) decreased the amount of irreversible binding of $^{14}CCl_4$ to liver microsomal lipids and reduced CCl_4-induced lipid peroxidation at 1 hr but not at 6 hr after administration of the antidotal drug. Cycloheximide did not alter the pentobarbital sleeping time of the rats nor did it interact with microsomes to give spectral changes. Cycloheximide did not alter microsomal cytochrome P-450 reductase activity. Administration of cycloheximide prior to CCl_4 did not prevent the CCl_4-induced decrease in cytochrome P-450 or glucose 6-phosphatase activity caused by the hepatotoxin, however it did prevent the CCl_4-induced polysome breakdown and it did attenuate the severity of the lesions caused by CCl_4 at ultrastructural level. The results are consistent with the view that the protective effects of cycloheximide may be indirectly or even unrelated to its effects on protein metabolism, but may instead be related to an effect on the metabolic activation of CCl_4 to CCl_3 free radical, the putative proximate toxicant.

An extension of this study utilized chloramphenicol as an inhibitor of protein biosynthesis to investigate its possible role in CCl_4 hepatotoxicity. Chloramphenicol administered to rats 24 or 72 hours prior to CCl_4 prevented liver necrosis without altering body temperature or the CCl_4 levels in the liver. Pretreatment with chloramphenicol reduced the covalent binding of CCl_4 to hepatic microsomal lipids, CCl_4 - induced lipid peroxidation and polysome breakdown.

F. The Effect of Whole-body Hyperthermia on the Disposition and Metabolism of Adriamycin. The effects of whole body hyperthermia on the disposition and metabolism of adriamycin were investigated in male New Zealand rabbits. Hyperthermia [rectal temperature, 42.3 ± 0.1° (S.D.)] was produced in non-acclimatized, unrestrained animals by placing them in a humidity (95%)-and temperature (34°)-regulated chamber. Normothermic controls (rectal temperature, 39.7 ± 0.1°) were maintained at ambient temperature and humidity. Adriamycin was administered i.v. to both groups over a 60-sec. period at a dose of 5 mg/kg, and arterial blood samples were removed serially at several times up to 60 min. Animals were sacrificed with pentobarbital, and bladder urine, bile, and tissue samples were removed and immediately frozen until analyzed fluorometrically.

In general, the rate of clearance of adriamycin from the plasma was similar in both groups; however, small but significant decreases in the distributive phase were observed in the hyperthermic group. Analysis of tissue concentrations of adriamycin, adriamycinol, and aglycones revealed significant increases only in skeletal muscle and duodenum of hyperthermic compared to normothermic rabbits. Urinary and biliary levels of adriamycin and its metabolites were not different in the two groups, but the percentage of total urinary products attributable to adriamycinol was significantly higher in the hyperthermic group. Adriamycin treatment resulted in significant reductions in plasma calcium and significant increases in plasma aldolase in both experimental groups, but other parameters including lactic dehydrogenase, serum glutamic oxalacetic transaminase, alkaline phosphatase, phosphorus, magnesium, potassium, sodium, chloride, carbon dioxide, blood urea nitrogen, creatinine, cholesterol, bilirubin, uric acid, and total protein remained within normal limits.

G. Studies on the Interaction Between α-tocopherol and Adriamycin in vivo. Various investigators have attempted to correlate biochemical alterations in animals with the pathogensis of adriamycin cardiotoxicity. Recently it has been reported that D-α-tocopherol pretreatment alters the lethality of adriamycin in mice, and prevents adriamycin-induced cardiomyopathy. As part of our continuing studies to understand the mechanisms by which antineoplastic drugs elicit toxicity we have investigated the effects of α-tocopherol on the pharmacokinetics, disposition, metabolism and lethality of adriamycin in CDF_1 mice.

Animals were treated with either α-tocopherol, olive oil or saline solution, ip. 24 hours prior to ^{14}C-adriamycin administration, 15 mg/kg, also ip. α-Tocopherol and olive oil caused similar alterations in blood and tissue levels of ^{14}C-adriamycin-derived radioactivity. Blood levels of adriamycin-derived radioactivity in animals pretreated with olive oil or α-tocopherol were approximately twice those of saline controls between 3 and 15 min. after injection. At 30 and 60 min., blood levels did not differ in the three groups. At 60 min. after adriamycin injection, levels of activity in liver, kidney, lung, and heart were significantly higher in the α-tocopherol and olive oil groups than in saline controls, thus reflecting differences in peak blood levels.

The following pharmacokinetic parameters for adriamycin were increased by α-tocopherol treatment: $t_{1/2}$ α, k_a, k_{el}, and C_p^o. Conversely, α-tocopherol decreased k_{12}, k_{21}, Vc, Vd ss, and V_t for adriamycin. Chromatographic separation and quantitation of adriamycin revealed no significant differences in hepatic or cardiac levels in any group. However, renal levels of adriamycin and several metabolites were elevated in both the α-tocopherol and olive oil groups, and the adriamycin concentration was higher in the lungs of animals in the olive oil group. In general, the effects of α-tocopherol and olive oil on the metabolism of adriamycin did not differ from saline controls.

Animals treated as described above lost weight at similar rates with maximum losses (25%) in the survivors at 7-14 days. Fourteen days after adriamycin treatment there was >50% mortality in both the olive oil and saline groups, but only 5% mortality in the α-tocopherol group. However, by 28 days, 30% of the animals in the α-tocopherol group were dead compared to 60% in the other 2 groups, and at 60 days, mortality was 80 ± 5% in all 3 groups. There were no deaths in a group of animals treated with a single dose of α-tocopherol alone.

Thus, the efficacy of α-tocopherol against the lethal toxicity of adriamycin in mice is exceedingly time-dependent. Two weeks after adriamycin injection, there was a dramatic protection by α-tocopherol relative to saline controls (50% mortality vs 5%) but by 9 weeks, mortality rates were not different in the two groups. Our studies suggest that the major effect of α-tocopherol on the lethal toxicity of adriamycin is to delay rather than prevent it.

H. Effect of Obesity on Drug Metabolism in Rodents. Literature reports have suggested that differences in drug response between obese and non-obese patients might be related to differences in microsomal drug metabolism. Further, differences in chemical carcinogenesis between normal and obese rodents, and human epidemiological data suggesting different tumor incidence in obese and lean humans suggest a possible metabolic relationship between carcinogenesis and obesity. We therefore investigated the activity of drug metabolizing enzymes in genetically obese rodents. Obese male rats have *in vitro* hepatic drug metabolizing enzyme activities that are approximately 30% those found in genetically normal animals. Cytochrome P-450 levels are 65% of levels in normal rats. In obese female rats only NADPH cytochrome c reductase and aniline hydroxylase activities were lower than in normal female rats. Enzyme activities in obese females were routinely greater than in the genetically obese males. Hexobarbital sleeping times in obese males were nearly twice those in normal males and both groups had similar blood levels of hexobarbital at awakening. In male weanling obese rats, cytochrome P-450 levels were equal to or greater than in non-obese controls, aminopyrine demethylase activity was 80% of control activity, and UDP glucuronyl-transferase activity was higher than in controls. Adult obese male rats responded like the control lean rats to an enzyme-inducing regimen of methylcholanthrene (3-MC). Obese male rats, however, had an exaggerated sensitivity to the effect of phenobarbital (PB) and a decreased inductive response to PB. All obese male rats treated at 75 mg/kg/day died after two treatments. Half of the obese rats treated at 60 mg/kg/day for four days died by day 4. Forty-five mg/kg/day for four days produced prolonged sedation but no deaths and 30 mg/kg/day for four days produced no overt effects but minimal induction (15-20%). Forty-five mg/kg/day for three days produced a slight increase in enzyme activity (50%). Normal animals at the same dose show the expected 2-4 fold increases in enzyme activities. Weanling fat rats demonstrated variable induction after 4 daily doses of 45 mg/kg PB, with 30-50% increases in most parameters and a 100% increase in aminopyrine demethylase activity.

Endogenous drug metabolism in obese male mice was variable, with NADPH cytochrome c reductase, aminopyrine demethylase, and PABA acetyl transferase activities nearly equal to normal animals, but aniline hydroxylase, cytochrome P-450 and UDP-glucuronyl transferase activities 30-50% of normal values. Obese male mice responded normally to induction with 3-MC.

I. Transport of Model Compounds and Antineoplastic Agents Across the Rat Peritoneum. We tested the feasiblity of using intraperitoneal administration of antineoplastic agents containing higher than therapeutically active doses, the hypothesis being that the entire peritoneal surface would be bathed in drug solution while systemic absorption and its potentially lethal side-effects would be minimized. This mode of treatment could serve as adjunct therapy to surgical resection in the earlier stages of ovarian carcinoma which are characterized by microscopic intraperitoneal metastases.

The transport of various model compounds and antineoplastic agents across the peritoneum of the rat was investigated to assess the influence of three major parameters on their absorption into the systemic circulation: 1) molecular weight; 2) the dissociation constant (pKa); and 3) the lipid water partition coefficient (K). Sprague-Dawley rats were injected intraperitoneally with radiolabeled model compounds (25) and antineoplastic agents (15) in large volumes of normal saline (50 ml). After one hour, the peritoneal fluid was removed and the absorption and tissue distribution of the compounds determined.

The 9 neutral model compounds tested ranged in molecular weight from 18 to 2 million; their peritoneal absorption varied from 69 to 2% respectively. Five compounds were investigated with variable K values in heptane (0.001-10); their absorption ranged from 57-96% respectively. A series of 10 acids and bases covering a wide range of pKa values were examined; for the acids, absorption increased with increasing pKa values, while for bases the reverse appeared to be true. The absorption characteristics of 15 antineoplastic agents were determined; their absorption ranged from 9% for L-asparaginase to 91% for hexamethylmelamine. A correlation was made to their respective molecular weight, pKa and K values. From these results recommendations were made as to which agents, i.e., those having minimal absorption, should be employed in this adjunct mode of therapy for ovarian carcinoma.

Further experimental work has shown that the transport characteristics of the peritoneal membrane apparently do not change during an 8 hour course of therapy. Eight hour absorption of both 5-fluorouracil (5-FU) and adriamycin followed rates of absorption predicted by the 1 hour data, and organ distribution was found to be similar to that demonstrated after one hour of exposure. Fluid transport out of the peritoneal cavity was found to be highly dependent on the vehicle. In contrast to 0.85% NaCl, Inpersol (1.5% dextrose solution utilized in clinical peritoneal dialysis) results in intraperitoneal volumes that are sustained or increased by 20% over an 8 hour time course. This change in fluid shift, however, did not significantly alter the absorption of 5-FU or adriamycin.

J. Disposition of Cis-diamminedichloroplatinum (CDDP). In conjunction with the Biomedical Engineering and Instrumentation Branch, Division of Research Services, we have investigated the disposition of CDDP following its intravenous injection (1 mg/kg) in beagle dogs, sharks and rats. Plasma levels of platinum in dogs showed a distinctly biphasic clearance pattern with estimated half-times of about 20 minutes and nearly five days. During the first four hours after treatment, plasma levels fell by 90% and 60-70% of the administered dose was recovered in the urine. Platinum was still detectable twelve days after treatment, with no significant change in plasma concentration after the fourth day. Initial concentrations of platinum were highest in organs of excretion, in gonads, spleen and adrenals but remained significantly elevated only in kidney, liver, ovary and uterus, where tissue:plasma ratios of 3-4 were maintained for as long as six days.

Following i.v. administration of CDDP to male rats, plasma platinum levels also had a biphasic half-time with an initial phase of about 40 minutes and a long phase of two days. Small quantities of platinum were recovered in urine during the first 48 hours post-treatment. Organ distribution was similar to that in the dog, with initial and persisting levels of platinum found in liver, kidney and gonad.

The dogfish shark exhibited the slowest rate of clearance from the blood, with an initial half-life of nearly 70 min. and a long phase half-life of about 10 days. Six days after treatment, only 10% of the administered dose had been recovered in the urine. Shark kidney and uterus contained large and persisting amounts of platinum, but the liver in contrast to the other two species, contained only small amounts of platinum.

In a similar protocol which minimizes renal toxicity by prolonged hydration, plasma decay curves of platinum in hydrated and normal animals were found to be not significantly different.

K. Accumulation of Drugs by Lewis Lung Tumor. The Lewis Lung (LL) carcinoma is a pulmonary tumor of spontaneous origin, propagated in mice by im or sc implantation. Twenty days after implantation the solid tumor weighs approximately 6 g, or 25% of the animal's body weight. Since LL is of pulmonary origin we investigated the hypothesis that basic drugs, which are accumulated by the lung may also be taken up by the tumor. At 6, 10, 15 and 20 days after implantation of tumor, groups of mice received ^{14}C-imipramine (10 mg/kg, ip). During the first few hours following imipramine administration, contrary to other tissues, the tumor showed an apparent absorption phase which was more pronounced in large tumors. The decay of radioactivity in plasma, lung, liver and kidney was similar in control and tumor-bearing mice. However, after reaching peak levels, the clearance of radioactivity from the tumor decreased with increasing size of the tumor.

The metabolic profile of imipramine was similar in the same tissues from control and tumor-bearing mice but was different in different tissues. In lung and tumor, 55-70% of the total radioactivity was associated with unchanged imipramine, whereas in the liver only 12-16% was imipramine, the rest being 2-hydroxyimipramine (35-55%) and desdimethylimipramine (7-14%). Overall, the presence and growth of LL tumor had little effect on the disposition of imipramine in male BDF_1 mice, but there was evidence to suggest that animals bearing the tumor for 20 days had slightly altered metabolism of imipramine.

The accumulation of basic drugs was also compared in mouse lung slices and 10 day LL tumor slices. Lung and tumor showed similar accumulation curves for imipramine; both tissues accumulated serotonin to a similar degree after 2 hrs incubation but the uptake by tumor was linear with time while that by the lung exhibited saturation kinetics; accumulation of paraquat by lung was linear with time but the tumor failed to accumulate the drug. Both serotonin and paraquat are actively accumulated by lungs. This work is being continued.

L. Accumulation of Xenobiotics by the Lung. Evidence has been presented over the last decade that basic drugs (pKa >8.5) are concentrated and retained by the lungs of mammalian species. However, recent work in our laboratory suggests that the accumulation of such xenobiotics is more complicated than suggested by previous data. We have investigated the pulmonary retention of a model basic drug (imipramine) in several species and found both a species and dose dependency. Twelve hours after administering ^{14}C-imipramine (50 mg/kg, ip) to mice, rats, guinea pigs, and rabbits, only rats and rabbits exhibited significant accumulation of radioactivity in the lungs relative to other tissues. For the rat, tissue:plasma ratios for lung, liver and kidney were 45, 18 and 12 respectively. In mice and guinea pigs, the liver had the highest tissue:plasma ratio (19 and 5 respectively). Lung:plasma and kidney:plasma ratios were both around 2 in mice and 5 in guinea pigs. In addition to this species difference, we have demonstrated that the accumulation of imipramine in rat and rabbit lung is also dose-dependent. In rats twelve hours after administration of ^{14}C-imipramine (10 or 50 mg/kg, ip) the lung and liver:plasma ratios of radioactivity were 3 and 14 for 10 mg/kg and 46 and 18 for the 50 mg/kg dose. The apparent threshold dose at which pulmonary retention of imipramine occurs is ∿30 mg/kg. Mice, guinea pigs, and rabbits showed no pulmonary retention of radioactivity after administration of 10 mg/kg, ip. Although imipramine is not metabolized by pulmonary tissue, it undergoes extensive metabolism by the liver. Hence, it is possible that the species difference in pulmonary accumulation of the drug may be related to differences in hepatic metabolism.

Experiments with lung slices indicate that the rat lung may be able to concentrate imipramine to an extent of 30-40 fold over the medium. At tissue:medium concentrations exceeding 30-40, the concentration gradient may be such that outward diffusion counterbalances active accumulation. However, the rate of efflux of imipramine from lung slices is independent of the amount accumulated. Thus, the amount the of drug left in rat lung after a given time of efflux is proportional to the initial concentration attained.

The lung slice technique has also been employed to investigate the accumulation and retention of paraquat, a herbicide which is actively transported into rat lung. Although imipramine can inhibit the pulmonary uptake of paraquat the two drugs do not have identical uptake mechanisms or receptor/storage sites. The accumulation curves of the drugs are different. Imipramine inhibits the uptake of paraquat but paraquat increases the uptake of imipramine. Paraquat does not readily efflux from rat lung slices whereas imipramine decays to approximately 50% of its initial level after 2 hours and neither drug affects the efflux of the other. In addition, lung slices obtained from rats treated with paraquat (100 µmol/kg, iv) 16 hours previously showed a 60% decrease in paraquat accumulation whereas accumulation of imipramine was unaffected. These effects were observed in the absence of edema, or changes in sucrose and inulin spaces.

M. Effect of Vehicle on Paraquat Toxicity. It has been reported that paraquat administered in water to mice is more toxic than when given in saline. We have extended this observation and have demonstrated the effect both ip and sc. Although the degree of protection is dependent upon the concentration of saline there appears to be a threshold level of saline above which no further protection is afforded. After administering paraquat in saline (45mg/kg, ip) there was an apparent decrease in the absorption of paraquat compared to when it is administered as a solution in water. Although there was no difference in the urinary excretion of paraquat over 12 hrs after an ip dose, the plasma clearance of paraquat given iv (45 mg/kg) was slower when dissolved in water than in saline. There were differences between the 2 vehicles in the distribution of paraquat after ip administration. When given in water, the liver had higher paraquat content 15 and 30 min. after administration, and after 24 hrs both the kidney and lung had considerably higher concentrations of paraquat than when administered in saline.

It may be that the protection that saline affords against paraquat toxicity is a result of a combination of altered parameters, namely decreased absorption, increased plasma clearance (renal clearance) and consequently altered distribution.

N. Effects of Schedules of Administration of Bleomycin on Therapy of Lewis Lung Carcinoma and Pulmonary Toxicity in Mice. There are suggestions in the clinical literature that continuous intravenous infusion of bleomycin, or more frequent injections of lower doses, may result in an improved therapeutic index as compared to conventional weekly treatment by i.v. bolus injection. We treated BDF$_1$ mice bearing Lewis Lung carcinoma with three schedules of bleomycin: 10 mg/kg twice weekly versus 1 mg/kg and 2 mg/kg ten times weekly. Two mg/kg ten times weekly produced a 34% ILS, significantly different from controls. One mg/kg ten times weekly produced a 27% ILS, which was also significant. However, the more intermittent schedule, 10 mg/kg twice weekly, did not result in significantly increased survival over controls. There was no significant difference in lung toxicity produced by 10 mg/kg twice weekly for four weeks versus 2 mg/kg ten times weekly for four weeks, as measured by total lung collagen content and the following morphometric parameters: number of intraalveolar macrophages and leukocytes, total pulmonary cell count, mean alveolar wall thickness, and percent consolidation of lung parenchyma. We conclude that, at equal total doses of bleomycin, more frequent injection of smaller individual doses of drug is more effective in treatment of Lewis Lung carcinoma than higher, more intermittent doses, while the pulmonary toxicity of the two schedules is approximately the same.

Further experiments have compared these schedules of bleomycin injection with continuous infusion of bleomycin subcutaneously (s.c.) in mice via the Alzet^R minipump. Continuous infusion was significantly superior to equivalent doses of bleomycin administered by intermittent injection in reducing the size of Lewis Lung carcinoma. Effects of these schedules upon pulmonary toxicity were compared using whole lung collagen content. A total dose of 40 mg/kg of bleomycin s.c. produced significant increases in lung hydroxyproline levels compared to saline controls, when the bleomycin was administered by injection twice or ten times weekly, but no increases when given as a continuous infusion for seven days. Thus, continuous infusion reduced the pulmonary toxicity of bleomycin and increased therapuetic efficacy against Lewis Lung carcinoma in mice. Since randomized comparisons of continuous infusion versus intermittent injection of bleomycin have not yet been done in man, this is the first rigorously controlled experimental evidence that continuous infusion of bleomycin actually does improve the therapeutic index of the drug.

In the past several months, we have developed a reversed phase, high pressure liquid chromatographic assay for bleomycin and its major metabolites, utilizing both C-18 and phenyl-bonded columns, and either heptane or pentane sulfonic acids as paired-ion reagents. This assay has enabled us to measure activities of bleomycin hydrolase in rat and mouse tissues. This enzyme has been reported to hydrolyze a terminal amino group from the β-aminoalanine moiety of bleomycin thus abolishing the antibacterial and antitumor effects of the drug. Levels of bleomycin hydrolase were reportedly low in lung tissue, skin, and in a mouse tumor sensitive to bleomycin. We are further investigating the properties of this enzyme, especially in relationship to its inducibility, and to the potential correlation of low activities in tumors with therapeutic activity of bleomycin.

O. Drug Metabolism in the Nude Athymic Mouse. Present indications are that the heavy reliance that has been placed in the past on the L1210 leukemia and B16 melanoma systems in screening for potential new antineoplastic compounds will be supplanted in part by explants and transplants of human and animal tumors. The host organism for these tumors will be the nude, athymic mouse whose aberrant immunologic system does not reject these heterologous tumors. Knowledge of the ability of this mutant mouse strain to metabolize drugs is therefore of obvious importance.

Liver microsomal and supernatant fractions from male and female athymic nude mice (nu/nu) of the NIH Swiss background were studied for their ability to metabolize drug substrates *in vitro*. Controls were homozygous NIH Swiss mice (++) and mice heterozygous for the athymic gene (nu/+). The following enzyme activities were higher in the male athymic animals than in controls: NADPH cytochrome c reductase, ethylmorphine and aminopyrine N-demethylase, 'native' UDP-glucuronyltransferase and glutathione S-aryltransferase. No difference in activity between the athymic mice and controls was seen with UDP-NAG-activated UDP-glucuronyl transferase, PABA N-acetyltransferase, aniline hydroxylase, or in the amounts of cytochrome P-450. Liver:body weight ratio was slightly higher in the athymics than in controls (5.9 vs 5.2%) but microsomal protein yield was less (26.7 vs 32.9 mg/g).

In the female, only the N-demethylase pathway with both substrates was significantly higher in the athymics relative to controls. Athymic mice responded quantitatively and qualitiatively like the controls to pretreatment with either phenobarbital or 3-MC. Enzyme activities from heterozygous mice segregated into 3 separate categories relative to the controls and to the homozygous recessives. The data are consistent with a hypothesis that MFO activity may be passed genetically as a single allele that undergoes traditional dominant-recessive expression.

Significance to Biomedical Research and the Programs of the Institute: As stated under objectives, drug interactions are known to profoundly influence the disposition of drugs and thus may determine whether a normal therapeutic dose of drug will become either a sub-threshold or toxic dose. The significance of this knowledge to cancer chemotherapy, particularly in the era of combination drug administration, cannot be overemphasized. The studies on peritoneal transport have great potential in the treatment of ovarian cancer.

Proposed Course: The projects being pursued are only partially complete. In addition to examining in more detail the influence of antineoplastic agents upon the metabolism, distribution, and binding of drugs and their toxicity, we envision studying the metabolic fate of antineoplastic agents. Pharmacokinetic studies of the dogfish shark model have only begun and will continue in greater detail, particularly for those anticancer agents just entering clinical trials.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06143-01 LT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Selected Transport, Metabolic and Toxicologic Characteristics of Antineoplastic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.8	2.3	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. To study the transport of anticancer drugs and model substances particularly as they apply to biliary and peritoneal systems.
2. To apply current analytic methodology to the quantification and identification of antineoplastic drug metabolites, especially as such biotransformations occur in the course of biliary and peritoneal transport experiments.
3. To apply objectives 1 and 2 to a wide variety of mammals and aquatic species, with the goal of testing pharmacokinetic models in a wide spectrum of species.
4. To scale-up pharmacokinetic models from the experimental data base and to provide pharmacokinetic support for the Phase I Clinical Trials ongoing in the Clinical Center.
5. To assess the accuracy of qualitative and quantitative methodology used in toxicologic testing of oncolytics in animals, to predict for toxic events occurring in man.

Project Description:

Objectives:

1. To study the transport of anticancer drugs and model substances particularly as they apply to biliary and peritoneal systems.
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5. To assess the accuracy of qualitative and quantitative methodology used in toxicologic testing of oncolytics in animals, to predict for toxic events occurring in man.

Methods Employed:

Standard analytic, biochemical, pharmacologic, pharmacokinetic and toxicometric techniques are employed.

Major Findings:

1. Biliary and Urinary Excretion of Adriamycin (ADR) in Anesthetized Rats: Although the pharmacokinetic properties of ADR have been investigated in a variety of animal species, including man, no detailed information on the mechanisms underlying its biliary and urinary excretions are available. To better elucidate its elimination pathways, we studied the biliary (renal-ligated animals, Group A) and biliary and urinary (intact animals, Group B) excretions of ADR and ADR-metabolites following i.v. injection of ADR to anesthetized rats at different doses (5-40 mg/kg). Protection of all samples from fluorescent light greatly improved (2-fold) recoveries compared with previously published assays. We also evaluated the biliary and urinary excretion of ADR (intact animals, Group C) administered after an i.v. injection of 50 mg/kg of tetracycline (TTC). The results obtained showed that ADR is rapidly cleared from plasma, concentrated in tissues and eliminated through the biliary and urinary routes mostly as parent compound (75-80% of total fluorescence). When ADR was given at doses ranging from 5 to 40 mg/kg (Group A), irrespective of the administered dose, 35-40% of the injected dose was recovered in bile (as measured by total fluorescence) throughout a 10 hr collection period. In group B, when ADR was given at 5 and 20 mg/kg, 30-35% of the injected dose was detected in bile and 5-8% in urine in this same time interval. Adriamycinol and ADR-conjugates were found as major metabolites in bile (4.5% and 2.7%) and urine (0.8% and 0.5%), but were not detected in hepatic and renal tissue at the end of 10-hr experiments. Less polar metabolites, including aglycones, were detected in bile (0.4%) and urine (0.2%) only in the first 4-hr collections,

although they were observed in liver and kidney at the end of the experiment (10 hr). When ADR was given following administration of TTC (Group C), the total fluorescence determined in bile (12%) and urine (0.3%) in the first hour collection was significantly lower than that observed when ADR was given alone (16.5% and 2.6% of the administered dose in bile and urine respectively). Despite these differences, the plasma clearance curves were substantially the same. In TTC pretreated animals, although renal levels of ADR-total fluorescence were significantly higher, the heart and liver did not show any significant difference. The present findings suggest that: (1) ADR is minimally metabolized in anesthetized rats; (2) ADR and ADR-metabolites are excreted 4-fold more by the liver than by kidneys, and (3), TTC pretreatment inhibits partially the biliary and extensively the urinary excretion of ADR.

2. Comparative Pharmacologic and Toxicologic Information. The primary reason for doing animal pharmacologic and toxicologic studies is to increase the predictability of the effects of a drug in man, to as close to 100% as possible. At the present this cannot be achieved and some of the reasons for these limitations are under investigation in this project. Some have called this area of drug development the "comparative data game". The traditional game plan has been to try to find the "species most like man". We have suggested an approach which first asks how can we rapidly and economically accrue the most important pharmacologic disposition and pharmacokinetic information needed by a drug developmental program. Since the ultimate action of a drug is due to its interaction with a receptor, it is assumed that the receptors of different species are similar and that such interactions will produce the same biologic response if the drug can arrive at the receptor in sufficient quantities. Thus, major species differences in pharmacologic or toxicologic responses arise from those events preceeding or decreasing drug-receptor reactions, i.e., sites of loss of a drug.

a. Comparative Pharmacology. We can now ask what pharmacologic disposition information is of most value during clinical trials? Plasma and other indices of biologic half-life tell the physician when the next dose of a drug can be administered. Easily derived from plasma $t_{0.5}$ values is the volume of distribution, V_D, which can yield valuable information even before one knows about the extent of binding, metabolism and excretion. The degree of plasma binding, rates and major pathways of metabolism, unusually high tissue levels, and relative dominance of urinary versus biliary excretion, round out the most important data elements needed by a drug program.

Most drug testing is done in rats and dogs and this self-perpetuating choice is largely based on convenience and availability rather than on hard science. The major basis for continuing to use any particular species for any drug developmental program is if it predicts for analogous effects seen in man. One type of inductive reasoning is the argument by analogy in which things that resemble each other in many ways are considered likely to resemble each other in other ways. The strength of this philosophic process is proportional to the number of facts accumulated which are relevant to the actions being studied. Thus, if a variety of species are tested and they all show a particular response, then it is highly likely that the human species will also show this same response.

Employing the general procedures developed by Rall and Zubrod in 1962, and the pathfinding pharmacokinetic extensions of such methods by Zaharko, Dedrick, and Oliverio in 1972 to other marine species, we recently summarized an ongoing study of more than 2 dozen anticancer drugs in the dogfish shark. For example, we found that initial plasma half-time significantly differed in mammals compared with shark values for only 2 drugs (7%), inosine dialdehyde and the Pt compound. There were seven cases where the elimination phase half-times differed considerably between species but qualitatively the shark still predicted in the correct direction, i.e., for long $t_{0.5}$ values. In most cases (16 of 20), the shark data could distinguish whether the V_D was less than, equivalent to, or greater than body water. For 28 drugs, data were available regarding the relative dominance of the urinary vs biliary excretion routes in terrestrial mammals (usually man) and in the dogfish. In every case the shark validated the results found in other species! Metabolism data were available only for about one-half the drug studies and for these there were no differences between species in terms of those which could be classified as extensively, moderately or poorly metabolized. Similarly, while plasma binding studies are incomplete, the shark invariably showed somewhat less drug bound than mammals, but nonetheless, at least moderate binding occurred for all anticancer drugs investigated. CNS penetration was readily assessed in the shark. Since comparative data were available one could classify slight, moderate and rapid rates in higher animals with these values seen in the fish. Results in the shark have been correlated to therapy of mouse brain tumors. In general, clearance rates were lower in fish than in mammals, but this was not only anticipated and could be accounted for in pharmacokinetic modeling, but might be exploited to study such processes which occur too rapidly in mammalian species. Of course, as with the validation of any model, some further work should be done. On the other hand, as long as toxicologic testing continues to be done in mammals, there seems to be no reason not to emphasize the shark for pharmacologic disposition studies since its predictive value at least equals that of any other species studied.

b. Comparative Toxicology. Acute toxicity tests have been part of our protocol for a number of years. Such tests have a two-fold role: first, to provide a basis upon which to design further toxicologic testing; second, to learn something about the way in which the drug is acting as a poison. Some workers have proposed a larger role for mouse LD_{50} data, i.e., to establish starting doses and dose progressions for Phase I clinical trials. The hazards of employing LD_{50} data in this manner have been pointed out in a survey completed this year. One major problem with such data is that IND files such as ours, will often contain a number of LD_{50}'s for the same drug schedule. We have been able to account for some variability from differences in strain, route of administration and vehicle but there are apparently other unknown factors of variability in LD_{50}'s. Even where these three variables are controlled, of the 16 drugs tested by the same regimens, 50%

of the comparisons lacked parallelism of the probit dose response lines. In basic toxicology, this is very important in that such lack of parallelism means either of two things: 1) the test populations are from groups too heterogenous in response to truly compare; or 2), if one can compare the groups, then the drug is killing by a different mechanism in each group. Furthermore, it was noted that several drugs had LD_{50}'s which differed considerably from each other, e.g., by up to 245%. Such acute studies yield only quantitative information and a better way of discovering qualitative toxicologic aspects of a drug is to go beyond "counting dead animals". Thus, our LD_{50} study presently includes an added dimension; limited clinical chemistries and hematology, as well as histopathologic evaluation of major tissues, are done in order to define the target organs affected by the drug.

We recently increased the sample size and updated the Schein report of 1970. In this new compilation instead of attempting to assess the predictiveness of animals to forecast all human toxicities, focus was placed on the predictiveness for principal clinical toxicities as defined primarily from literature citations, and the annual reports to the FDA of the Cancer Therapy Evaluation Branch, NCI. Animal data were collected from dogs and monkeys in accordance with the LT protocol. It should be noted that as the "state-of-the-art" has changed, some animal toxicities missed could have been due to deficiencies in earlier procedures or in the awareness level of the preclinical scientists. The major point of this analysis was to see if the routinely used procedures detected the indicated signs.

For 44 anticancer drugs, there were from 1 to 6 principal clinical organ toxicities. As has been noted on many previous occasions, most drugs of this class caused major GI and hematologic toxicities (73% and 80%, respectively). Cardiovascular aberrations were present for 7 drugs (16%) while toxicities to other sites occurred with the following frequencies: hepatic (18%); integumentary (18%); injection site (9%); nervous system (24%); respiratory (4%), and urinary (11%). The real gain of including a second species, in this case the rhesus monkey, should be considered. It was noted that of the total of 120 reported toxicities, there were only two instances, or 1.7%, of principal toxicities, hematologic in both instances, that occurred in the monkey but not the dog. The dog provided, therefore, virtually all of the predictiveness.

Regarding the animal predictiveness of adverse effects, there were excellent true positive correlations for the following individual organ systems; hepatic and respiratory (both 100%); hematopoietic (92%), nervous (91%); GI (88%); urinary (80%) and injection site (75%). Other organ sites such as cardiovascular, integumentary and special senses remain a problem in that validations ranged from 38-50%.

3. Intraperitoneal Cancer Chemotherapy. The feasibility of using large volumes to administer, by the intraperitoneal route, doses of antineoplastic agents greater than therapeutically active doses, has been studied. The hypothesis is that the entire peritoneal surface is bathed in drug solution

while systemic absorption and potentially lethal side-effects would be minimized. This mode of treatment could serve as adjunct therapy to surgical resection in early stages of ovarian carcinoma which are characterized by microscopic intraperitoneal metastases.

The transport of various model compounds and antineoplastic agents across the peritoneum of the rat was investigated to assess the influence of three major parameters on absorption of drug into the systemic circulation: 1) molecular weight; 2) the dissociation constant (pK_a); and 3), the lipid-water partition coefficient (K). Sprague-Dawley rats were injected intraperitoneally with radiolabeled model compounds (25) and antineoplastic agents (15) in large volumes of normal saline (50 ml). After one hour, the peritoneal fluid was removed and absorption and tissue distribution of the compounds were measured.

From these results recommendations were made as to which agents should be employed in this adjunct mode of therapy for ovarian carcinoma. Two Phase I clinical protocols, MTX and 5-FU, are underway to evaluate this technique for treatment of ovarian carcinoma.

LT continues to provide analytic and pharmacokinetic modeling support for these trials being conducted in the Clinical Center. We are also extending the basic research dimension of this project in order to provide a foundation for using other drugs and alternative vehicles, for example, which might favorably change the rates of peritoneal transport by manipulation of the osmotic gradients. Other preclinical studies involve peritoneal fluid volume monitoring, lymphatic flow involvement in peritoneal fluid loss, and the use of hydrogen polarography to monitor peritoneal blood vessel flow.

BEIB has a unique role in this project, namely that of assisting prospectively in the design of appropriate preclinical experiments, a priori scale-up to human clinical trials, and facilitation of feedback between the Clinical Center, BEIB and LT.

To summarize the present state of this project: the toxicologic and pharmacokinetic basis for this work has been established using the rat. Following pharmacokinetic modeling and scale-up to humans, the actual Phase I trials have been initiated using MTX and 5-FU. The preliminary results have shown that intraperitoneal administration of these drugs in large volumes of dialysis fluid, is practical.

Significance to Biomedical Research and the Programs of the Institute:

Analytic improvements of the ADR assay showed that the biliary transport is considerably more important in the rat, than has been previously reported. Thus, manipulation of biliary transport of ADR may afford a pharmacologic approach to altering the toxicity of this drug. The studies of the interaction of tetracycline with ADR point out the marked changes in excretion which can occur when both drugs are used. When patients receiving ADR are in need of antibacterial agents, those of the tetracycline-class should be avoided.

On the strength of the comparative pharmacologic and toxicometric data presented in this report and at an OD Seminar ((15 March 78), it was recommended that we propose major changes to FDA regarding use of alternatives to the traditional species studied and the use of shorter preclinical testing which substitutes the mouse for the monkey and includes some pharmacokinetic data in animals.

The intraperitoneal cancer chemotherapy studies have shown the excellent clinical advances which can be achieved by close coordination of bench and clinical scientists. The new procedure offers a second chance to the large percentage of patients who fail the traditional ovarian cancer protocols.

Proposed Course:

All three of these areas are in need of further study and the directions were alluded to in the subsections above.

Publications:

1. Jones, R. B., Myers, C. E., Hubbard, S. M., Dedrick, R. L., Guarino, A. M., DeVita, V. T., Jr.: Intraperitoneal cancer chemotherapy: preclinical and early phase I trials. Cancer Chemotherapy and Pharmacology. (in press), 1978.

2. Guarino, A. M.: Pharmacologic and Toxicologic Studies of Anticancer Drugs: of Sharks, Mice and Men (and Dogs and Monkeys). In Busch, H. and DeVita, V. T., Jr. (Eds.): Methods in Cancer Research. New York, Academic Press, Inc., Vol. 17, (in press), 1978.

SUMMARY REPORT OF THE LABORATORY OF CHEMICAL PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1977 to September 30, 1978

Efforts in the Laboratory of Chemical Pharmacology, DTP, DCT are concerned with conducting research, teaching and training, contract administration, and administrative and scientific support of the Developmental Therapeutics Program and the Division of Cancer Treatment

The research efforts in the Laboratory of Chemical Pharmacology are primarily concerned with elucidation of the pharmacological properties of new and established antitumor agents. These studies include their disposition and metabolism, mechanism of action, mechanism of resistance, and their embryotoxic, mutagenic, and carcinogenic effects. Studies are conducted with antitumor agents and other model foreign compounds in laboratory animals and in humans. Of special concern in the disposition studies of new antitumor agents is the tissue distribution of the compound, including its ability to cross the blood-brain barrier; in addition, the lymphatic absorption of the compound, its biotransformation and rate of elimination are evaluated in parallel with attempts to develop a model for its pharmacokinetic behavior. These studies require in many instances the development of new methodologies to facilitate pharmacologic studies of antineoplastic agents whose disposition and metabolic fate is not fully understood.

Other studies in the Laboratory of Chemical Pharmacology are concerned with the development of novel approaches to tumor therapy, the rational design of new antitumor agents, evaluation of chemotherapeutic agents in various model tumor systems, and characterization of the transport properties of the blood-brain-CSF system. Efforts are also underway to define the time-course of chemically-induced liver and ovarian tumors, and to develop methods for reversing the toxicity of antitumor agents.

During the past year a number of research projects were continued and several new projects were initiated. Some of the experimental findings are summarized below.

A 2.45 GHz microwave apparatus was designed and constructed to evaluate the effects of local hyperthermia on tumor growth. Adjustment of the temperature control resulted in elevated temperatures in the center of tumors which could be maintained to within ± 0.1°C without production of significant whole body hyperthermia. Hyperthermia was effective in the treatment of 3 (colon 38, glioma 26 and ovarian M5076) of the 6 tumors examined. Tumor growth in these systems was inhibited by as much as 85% and a few cures were obtained.

Studies on the antitumor activity of N-(phosphonacetyl)-L-aspartic acid (PALA) in several experimental systems were continued. PALA had minimal or no activity in all experimental leukemias and plasma cell tumors against which it was tested. It was very effective against several solid tumors (B-16 melanoma, Lewis lung carcinoma and M5076 ovarian carcinoma), in which it produced long term survivors in 20-70% of treated mice. A PALA-resistant subline of Lewis lung carcinoma (the tumor line most sensitive to PALA) was developed in order to study the biochemical mechanism of resistance. This subline is totally resistant to a dose of 200 mg/kg on 4 consecutive days. In contrast, with the sensitive Lewis lung carcinoma line, 8/8 cures are obtained with a dose of 100 mg/kg, and 5/8 cures are obtained with a 50 mg/kg dose. A comparison of sensitive and resistant lines revealed no differences in uridine kinase activity, indicating that resistance is not due to an enhanced salvage pathway. As compared with the sensitive line, the resistant subline showed a 10-30% increase in aspartate transcarbamylase activity, and 10-40% lower concentrations of radioactivity following a single dose of ^{14}C-PALA (100 mg/kg). However these differences are not sufficient to fully account for the total resistance of the subline to extremely high doses of PALA. Elucidation of the mechanism of resistance to PALA might provide useful information on the unusual spectrum of its anti-tumor activity as well as on its mechanism of action. Equally important is the clinical application of such findings, as they might predict which patients would benefit from PALA treatment and which drugs might be combined with PALA to exploit the biochemical lesion that results in PALA resistance.

The adenosine deaminase inhibitors, 2'-deoxycoformycin (2'-DCF) and erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) potentiate the antitumor activity of 9-β-D-xylofuranosyladenine (xylosyladenine) against leukemia P388, producing 30% long term survivors. The adenosine deaminase inhibitor 2'-DCF not only potentiates the cytotoxic and antitumor activity of various adenosine analogs, but it also exhibits immunosuppressive activity when used alone in several experimental systems. For example, guinea pigs were sensitized to mycobacterium and their delayed hypersensitivity skin test response to challenge with PPD was measured. Treatment with 2'-DCF either before sensitization or before recall significantly diminished skin test response. In another system monkeys were treated with 2'-DCF for a one week period. The response of lymphocytes from these aniamls, when tested _in vitro_ for responsiveness to a non-specific mitogen, was diminished compared to control animal cells and to their own cells when tested pre-treatment. Also, immunosuppressive activity was found using an allograft tumor test system in which LSTRA cells (a Moloney virus-induced leukemia in BALB/c mice) were transplanted subcutaneously into C57Bl/6 mice across an H-2 histocompatibility barrier. These studies support the concept that certain immunological functions can be suppressed _in vitro_ by adenosine deaminase inhibitors just as they can be restored _in vivo_ if adenosine deaminase is provided to humans deficient in this enzyme.

A synthetic polymer, divinylethermaleic anhydride copolymer (DIVEMA) was linked to methotrexate and the DIVEMA-MTX copolymer was evaluated for antitumor activity in two experimental murine tumors. The DIVEMA-MTX copolymer was evaluated against the L1210 ascites tumor as a system which is sensitive to MTX but not to DIVEMA alone. Preliminary results indicate an antitumor effect (ILS 200%) for DIVEMA-MTX greater than for MTX alone at optimal dose and schedule, or for equivalent doses of MTX plus DIVEMA. The DIVEMA-MTX copolymer was also tested for activity against the Lewis lung subcutaneous tumor as a system which is insensitive to MTX but sensitive to DIVEMA alone. The DIVEMA-MTX copolymer prolonged the life span of these mice by 40% over controls. At equivalent doses and the same schedules, MTX, DIVEMA, or DIVEMA plus MTX were ineffective. At higher doses, DIVEMA alone was effective in prolonging survival time, whereas higher doses of the MTX-DIVEMA polymer were toxic. This toxicity was a consequence of an alteration in MTX pharmacokinetics, resulting in prolonged plasma concentrations of MTX, when this drug was administered bound to the polymer.

Studies designed to elucidate the molecular basis of cytotoxicity of several anticancer agents were performed. In a comparative study, proflavine and 9-aminoacridine were found to bind to DNA as well as or better than 4'-(9-acridinylamino)-methanesulphon-m-anisidide (AMSA, NSC-141549) as determined by a fluorescence technique and by alteration of DNA thermal stability. Also, studies with DNA and RNA polymerases revealed that proflavine and 9-aminoacridine were better inhibitors of these enzymes than was AMSA. The results emphasize that many acridine compounds besides AMSA have considerable biological activity and, before a mechanism of action can be assigned to AMSA, the mechanism must be shown to be unique to AMSA and not shared by the general class of aminoacridines. o-AMSA is an analogue of AMSA in which the methoxy group in the meta position of the anisidide side-chain of AMSA is in the ortho position. In contrast to AMSA, o-AMSA was found to be inactive as an anti-tumor agent and approximately 10% as cytotoxic against L1210 cells in vitro. o-AMSA is as susceptible to thiolysis as is AMSA, binds to DNA, but is 50 to 75% less inhibitory to DNA-dependent RNA polymerase. A more detailed comparative investigation of the biological effects of o-AMSA and AMSA should yield valuable information as to the mechanism of action of AMSA. Present studies are examining the properties of DNA isolated from tumors and certain normal tissues following AMSA treatment, with special emphasis on the effects of AMSA metabolites on the integrity and biological properties of DNA.

Two new anthracenedione derivatives (NSC-279836 and NSC-287513) are active against a variety of animal tumors and are being considered for high priority development toward clinical trials. These compounds were tested for DNA interaction and possible effects on nucleic acid polymerizing enzymes as a prelude to a more detailed study of their mechanism(s) of selective toxicity. Each compound was found to bind tightly to calf thymus DNA, as anticipated from their multi-ring structure. Also, the template function of the DNA-drug complexes was found to be altered as determined by inhibition of DNA-dependent RNA polymerase activity. Enzyme activity was inhibited 50% at 20 μg/ml and 100% at drug concentrations greater than 100 μg/ml. This compares with concentrations of AMSA of 200-400 μg/ml necessary for 50% inhibition in the same system.

Analytical assay techniques were developed for 5 agents, thymidine, misonidazole, cis-dichlorodiammineplatinum, AMSA and PALA, to aid in their clinical evaluation and in studies of their pharmacokinetics in humans. Pharmacological disposition studies were initiated for four agents in laboratory animals (PALA, thymidine, misonidazole, and maytansine) and were continued for four agents (spirohydantoin mustard, cis-dichlorodiammineplatinum, AMSA and imidazopyrazole). Clinical pharmacokinetic studies are presently being conducted in patients receiving maytansine, PALA, AMSA, thymidine, cis-dichlorodiammineplatinum and gallium nitrate.

Plasma radioactivity levels following iv doses (100, 250 or 500 mg/kg) of ^{14}C-imidazopyrazole, to rats were multiphasic with a distribution phase over the first 60 mins ($T_{1/2}$ = 15-45 min) followed by a phase of very gradual decline over the next 5 hours ($T_{1/2}$ = 90 to 240 min). Plasma levels of radioactivity were proportional to dose at all time intervals with the slope of the slower second phase increasing slightly with decreasing dose. Radioactivity was distributed to all tissues; however, concentrations greater than those in plasma were found only in stomach, kidney, and liver at the 100 mg/kg dose, in stomach and kidney at the 250 mg/kg dose, and in stomach at the 500 mg/kg dose. The majority of the radioactivity was excreted in the urine, with 21.9% and 67.8% of the administered dose excreted by 6 and 24 hours, respectively. Fecal elimination was significantly less and accounted for 11.1% of total dose at 120 hours compared to 74.3% in the urine. Four hours after dosing (250 mg/kg, iv), 51% of the urinary radioactivity migrated on TLC plates as unchanged imidazopyrazole. The remainder of the radioactivity migrated in at least 9 different spots. A correlation between the sustained plasma levels and the long duration of action of imidazopyrazole was established. Drug concentrations equivalent to those found in plasma of rats between 15 mins and 8 hours after dosing inhibited the growth of L1210 cells *in vitro* by 80%. Concentrations of drug corresponding to plasma levels present *in vivo* between 8 to 20 hours after dosing resulted in greater than 50% inhibition.

Iodoantipyrine permeates brain capillaries very rapidly and is used to measure cerebral blood flow; therefore, it is an ideal reference compound for use in studies where the test drug or compound crosses the capillaries readily. The exchange of iodoantipyrine across the capillaries (blood to brain and vice versa) was studied and the brain to blood partition coefficient was determined. The distribution of spirohydantoin mustard (SHM) in the brain and systemic organs during intravenous infusion was studied in rats. Dimethylsulfoxide (DMSO), the vehicle used to keep SHM in solution, induced systemic hypotension and a fall in cerebral blood flow. Cerebral blood flow was measured simultaneously in these experiments using ^{125}I-iodoantipyrine. The rate constants for SHM exchange across the capillaries could not be accurately measured due to the progressive fall in blood pressure and cerebral blood flow during the experiments. However, a comparison with iodoantipyrine indicates that SHM crosses brain capillaries rapidly and that its brain to blood partition coefficient is in the range of 1.

Studies on the physiologic disposition of maytansine have been initiated. Rats given an iv injection of ^{3}H-maytansine excreted 61% and 6% of the administered radioactivity in 24-hour bile and urine, respectively. Less than 1% of the ^{3}H-activity was recovered in 24-hour thoracic duct lymph. Tritium activity disappeared from plasma with half-times of 54 minutes and > 24 hours for the alpha and beta phases, respectively. Preliminary results, using thin-layer chromatography, suggest that approximately 18% of the radioactivity in bile is attributable to unchanged maytansine. ^{3}H-maytansine was given as a 20-minute infusion to a patient with pancreatic carcinoma in whom biliary fistula had been established. Radioactivity in samples of blood, bile and urine was monitored at intervals for 20 hours after the end of the infusion. Bile contained 28% of the administered tritium activity, and 45% of the radioactivity was recovered in the urine. Tritium activity disappeared from plasma with half-times of 200 minutes and 16 hours for the alpha and beta phases, respectively.

PALA has recently entered Phase I Clinical Trials. A gas chromatographic assay for PALA was developed to obtain clinical pharmacokinetic data to aid in its clinical evaluation. Using this sensitive and specific assay, levels of PALA in serum and urine can be quantitated in the range of 0.5 μg/ml to 50 μg/ml. Analysis of samples taken from patients given doses of 100 mg/m^2 indicated peak PALA serum levels of 7-19 μg/ml. In two patients from whom sufficient samples were collected, the elimination half-lives were 3.9 and 6.3 hours, respectively. Analysis of urines from two patients indicated that 22% and 38% of the administered dose was eliminated in 24 hours. These values are considerably lower than those observed in the dog, in which 60% of the administered dose was excreted in the urine after 2 hours and 75% after 12 hours. Studies presently underway in the dog using C^{14}-PALA show peak serum levels of 450 μg/ml following a dose of 120 mg/kg (2400 mg/m^2) and a 10-fold decrease in serum levels in 2 hours. Levels of PALA in the CSF of the dog reached a maximum of 2.7 μg/ml, which is a cytotoxic concentration in vitro. Currently, three techniques (gas chromatographic analysis, radioactive assay using C^{14}-PALA, and inhibition of aspartate transcarbamylase activity) for determining levels of PALA in dog serum and urine are being compared to determine the reliability of each method and the extent to which PALA is metabolized. Information obtained from dogs relating to PALA disposition and possible metabolism will be utilized in further clinical pharmacologic studies.

Studies on the clinical pharmacokinetics of cis-DDP given as a 1 hour infusion have been completed. Platinum concentrations in urine and blood were determined by atomic absorption spectroscopy. Plasma concentrations of platinum exhibited a biphasic curve with an early fast decline, ($T_{1/2}$ = 23 min) followed by a slower second phase ($T_{1/2}$ = 67 hr). Urinary excretion data were used to calculate the plasma levels of non-protein bound platinum, and markedly different kinetics were noted for this fraction of the total plasma platinum. Protein bound cis-DDP was found to have minimal cytotoxicity against L1210 cells in vitro; thus, the concentration of active drug is directly related to levels of non-protein bound platinum. Levels of non-protein bound platinum demonstrated a biphasic curve, with a rapid decline having a $T_{1/2}$ of 8-10 minutes and a slower second decline with a $T_{1/2}$ of 45 minutes. Although the total plasma platinum concentration remains high for several days after cis-DDP infusion, the non-protein bound platinum falls to low levels within several hours of infusion. The urinary excretion rate parallels the non-protein bound platinum levels and most of the urinary excretion occurs in the first hours after infusion. Therefore, the danger of acute renal damage is probably significant only during the phase of rapid excretion, and, in contrast to methotrexate, renal failure developing days after infusion would not be expected to lead to cis-DDP toxicity.

Efforts to prepare phospholipid vesicles (liposomes) in which small molecular weight substances such as antitumor agents are encapsulated, are in progress. In preliminary studies, small unilamellar liposomes composed of phosphatidyl choline and ^{3}H-cholesterol (molar ratio 3:1) have been prepared which contain a trace amount of ^{14}C-glucose. Between 0.5-3% of the added glucose was encapsulated, and efforts are currently being aimed at improving this entrapment rate. Studies on the in vivo stability of such liposomes and their physiological disposition, including lymphatic absorption following intraperitoneal administration, are in progress. Such studies are a necessary prerequisite for further work on the lymphatic absorption of intraperitoneally administered antitumor agents encapsulated in liposomes of varying size.

A new autoradiographic technique has been developed which can measure focal changes in brain capillary permeability. The autoradiographic technique, using ^{14}C-γ-aminoisobutyric acid (AIB), can quantify up to a 100 fold increase and estimate up to a 1000 fold increase in the permeability of brain capillaries to AIB on the autoradiographs. It is possible to localize an increase in capillary permeability to brain regions as small as 100 to 200 microns in diameter on the autoradiographs. Color reconstructions of the autoradiographs enhance the appreciation of differences in AIB transport between neighboring brain regions and simplify the quantitative analysis. The blood to brain ECF (influx) rate constant for AIB is 5x10^{-4} and 2x10^{-3} min^{-1} in the normal rhesus monkey and rat, respectively. Autoradiographs of several brain lesions using ^{14}C-AIB have been prepared; they include the freeze lesion, X-irradiation injury, metastatic and chemically induced brain tumors, hyperosmotic blood-brain barrier opening, cerebral ischemia, and low energy microwave irradiation. Preliminary studies with the metastatic brain tumor model indicate that: (1) central tumor regions generally have very permeable capillaries, (2) some peripheral tumor regions demonstrate only small increases in capillary permeability, (3) in some regions where a sharp boundary between tumor and "normal brain" appears to exist on histological staining, an abrupt change in capillary permeability is seen. Preliminary studies with the X-irradiation injury model indicate that the first changes in capillary permeability may occur in cortex rather than white matter and these may preceed any histological evidence of capillary disruption or cerebral edema. The freeze lesion is sharply demarcated; there does not appear to be more than 1 to 2 mm between totally disrupted capillaries and "normal" capillaries. A two hour exposure to low energy microwave irradiation did not increase the permeability of brain capillaries to AIB.

New antitumor agents as well as clinically established drugs are being evaluated for embryotoxic and cytogenetic effects. Studies on the embryotoxic and cytogenetic effects of 2 inhibitors of the enzyme adenosine deaminase, 2'-deoxycoformycin (DCF) and erythro-9-(2-hydroxyl-3-nonyl)-adenine (EHNA), have been completed. DCF administered daily on days 7-11 of gestation did not possess marked embryotoxic activity at the dose (0.25 mg/kg) found optimal for enhancement of the antitumor activity of adenosine analogs against experimental animal tumors. Although a dose-related increase in the incidence of intrauterine mortality was noted at higher (0.375-2.0 mg/kg) dose levels of this drug, a statistically significant effect on the body weights or on the incidence of congenital malformations in the surviving fetuses was not found. Results with EHNA indicate that it also is without striking embryotoxic activity when given on days 7-11 of gestation at doses

(25, 50 and 100 mg/kg) known to potentiate the antitumor action of adenosine analogs.

Studies are in progress on the embryotoxicity of PALA. Daily treatment with PALA for 5 days during early gestation resulted in 100% intrauterine deaths in litters exposed to a dose (3 mg/kg/day) approximately 100-fold lower than that found optimal for increasing the survival of mice inoculated with B16 melanoma and Lewis lung carcinoma. Approximately 50% of fetuses survived daily PALA doses of 1.5 mg/kg, and a dose of 0.75 mg/kg was without embryolethal effects. The effects of single doses of PALA given on day 4,5,6,7,8,9 or 10 of gestation have also been evaluated. The peak of sensitivity to the embryolethal effects of PALA occurred on days 7 and 8 of gestation, when a dose of 10 mg/kg induced significant embryolethality and a 25 mg/kg dose induced 100% intrauterine deaths. PALA exerted teratogenic effects in the fetuses surviving doses of the drug ranging from 5-200 mg/kg given after day 5 of gestation. The peak of sensitivity to PALA-induced teratogenicity occurred on day 7. Thirty-five percent of live fetuses exposed to PALA on day 7 of gestation were malformed, whereas when this dose was given on day 6 or 8 of gestation, only 3.4% and 5.6%, respectively, of fetuses were malformed. The possibility of reversing the embryolethality of PALA by uridine is currently being evaluated. The best schedule tested to date involves 7 doses of uridine spaced 45 minutes apart, which decreased the embryolethality of PALA (25 mg/kg) from 100% to 65%. However, preliminary results suggest that a large proportion of the surviving fetuses are malformed. Efforts to improve the reversal of PALA-induced embryotoxicity with uridine are continuing. In addition, (acetyl-^{14}C)-PALA has recently become available, and studies on its physiologic disposition in pregnant and non-pregnant mice are underway.

The Salmonella test system, originally developed by B. N. Ames et al., is being used as a means of detecting the mutagenic effects of various chemical compounds. This system is based on backward mutation in a specific locus of the histidine operon in a histidine dependent Salmonella typhimurium mutant resulting in histidine independence. In addition, a forward mutation assay was developed with the histidine independent Salmonella mutant by selecting for 8-azaguanine resistance due to mutation in the structural gene for hypoxanthine-guanine phosphoribosyl transferase (HGPRT). With this system we are now capable of examining the frequency of the specific backward mutation as well as the general forward mutation (presumably detecting the entire set of possible mutagenic lesions in DNA caused by chemical compounds). Several antitumor agents have been examined and found to be mutagenic and this correlates with their known carcinogenic effect in animals. However, the antitumor agent PALA appears to be devoid of mutagenic activity.

The Salmonella typhimurium system was used to test the mutagenic activity in urine from the rat, mouse and guinea pig after administration of the potert urinary bladder carcinogen N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT). High mutagenic activity was found in urine from the mouse and rat, species known to be susceptible to the carcinogenic effect of FANFT. In contrast, no mutagenic activity was found in urine from guinea pig, which is resistant to FANFT. It is concluded that the Salmonella test system may be of value in detecting urinary mutagens and potential carcinogens as well as differentiating between individuals who are susceptible or resistant to urinary tract carcinogens. These studies have been extended to include examination of the mutagenic activity of: plasma and urine from patients on chemotherapy, urine from people living in areas with a high incidence of bladder carcinoma (Egypt), and extracts from transplantable tumors after treatment with antitumor agents known to be active or inactive against these tumors.

Rat and mouse ovarian aryl hydrocarbon hydroxylase (AHH) activity was measured and characterized in control and MCA-treated animals. Basal ovarian AHH activity was similar in C_{57}Bl/6N and DBA/2N mice; however, after MCA treatment an increase in gonadal AHH activity was observed only in B6 mice. The ovarian AHH activity was increased 2- to 3-fold in Sprague-Dawley, Lewis, and Brown-Norway rats following MCA treatment. The induction of AHH activity after MCA administration was the same in ovaries from weanling and adult Sprague-Dawley rats. Ovarian AHH activity was located in the microsomal fraction, required NADPH, and had a broad pH optimum between 7.0 and 8.8. Both carbon monoxide and antibody to cytochrome c reductase inhibited the ovarian AHH activity, suggesting that the hydroxylase is a cytochrome P-450-dependent monooxygenase.

A marked species difference between mice and rats was noted for MCA-induced oocyte toxicity. Seven days after a single ip injection of MCA, (80 mg/kg), 87% of the primordial oocytes were destroyed in the ovaries of B6 mice, 69% were destroyed in D2 mice, and none were destroyed in Sprague-Dawley rats. These data suggest that the oocyte toxicity of MCA in the mouse is related to the ovarian AHH activity, whereas no association is found between the ovarian AHH activity and oocyte toxicity in the rat.

The rodent ovary contains an enzyme system(s) capable of metabolizing polycyclic aromatic hydrocarbons to reactive electrophilic intermediates known to cause cytotoxicity, mutation, and cancer. This suggests that if the human ovary contains similar enzyme systems, metabolic activation of environmental chemicals might explain the earlier menopause in cigarette smokers and the higher incidence of ovarian cancer in industrialized areas.

From our survey of second tumors in treated cancer patients, we are accumulating evidence that Hodgkin's disease patients receiving treatment with the MOPP regimen, one component of which is procarbazine, are at increased risk of developing AML. Although a causal relationship between cytotoxic drug therapy and the appearance of AML in these patients has not yet been established, there is ample evidence that procarbazine is a potent carcinogen in mice and rats. In addition, the carcinogenic potential of procarbazine in 3 species of non-human primates is being evaluated. A total of 55 monkeys have received procarbazine for periods up to 11 years. Eleven of the 43 monkeys (26%) necropsied thus far have had malignant neoplasms, six of which were acute leukemia. Two monkeys developed osteogenic sarcomas, 2 monkeys developed hemangiosarcomas, and a single case of lymphocytic lymphoma was found. The average total dose of procarbazine received by the monkeys developing malignancies was 36.0 gm, the average duration of procarbazine treatment was 75 months. The 12 surviving monkeys in this study may represent a population at high risk for developing AML. For this reason, they are being utilized in hematologic and cytogenetic studies in order to determine whether a "pre-leukemic" phase is detectable prior to the development of frank drug-induced leukemia. For this purpose, sequential bone marrow samples from all of the monkeys receiving procarbazine are being examined for alterations in cellular morphology and for chromosomal aberrations. Since initiation of these studies, no specific chromosomal aberrations have been detected in bone marrow cells from the monkeys and no additional cases of acute leukemia have developed. However, a number of the toxic effects of procarbazine seen clinically are also noted in the monkeys, including vomiting and myelosuppression. Its most striking toxic effect, however, is on the reproductive system of the males. The majority of the adult males necropsied to date have had testicular atrophy with complete aplasia of the germinal epithelium.

N-methylnitrosourea (MNU) is under clinical trial in the USSR and has been reported to be effective in the treatment of lung cancer. We have evaluated its carcinogenic potential in three species of non-human primates. A total of 43 monkeys have received oral doses of MNU for periods up to 124 months. Six of the 18 monkeys (33%) necropsied thus far have had squamous cell carcinoma (SCA) of the mouth, pharynx and/or esophagus, and upper digestive tract lesions such as atrophy or dyskeratosis of the esophageal mucosa and esophagitis have been a consistent finding among the 18 monkeys necropsied to date. All but one of the animals receiving total doses of MNU exceeding 50 gm developed SCA after an average of 6 years of exposure. Many parallels were noted between the esophageal SCA observed in the present series of monkeys and human esophageal carcinoma, including the clinical manifestations of the tumor, its complications, its radiographic appearance and its morphology. The MNU-induced lesions of the oropharynx and esophagus of primates may therefore be a valuable model for the study of human esophageal carcinoma.

The potential carcinogenicity of adriamycin is also being evaluated. A group of 10 monkeys received an iv dose of drug (1 mg/kg) once each month for 23-28 months. At the end of the dosing period it was intended to hold the animals under observation for the remainder of their lives. However, approximately 2 months after the last dose of adriamycin 9 of 10 animals developed congestive heart failure. Histopathologic examination of cardiac muscle taken at necropsy revealed lesions characteristic of adriamycin-induced cardiomyopathy in humans, and in some cases these findings were confirmed by electron microscopy. In man, a cumulative adriamycin dose of 550 mg/m^2 has been associated with cardiac toxicity; non-human primates appear to be more sensitive to adriamycin-induced cardiomyopathy as the monkeys in the present study had received an average cumulative dose of 310 mg/m^2 (range 276-336 mg/m^2). Moreover, an additional finding at necropsy of one of the animals dying of congestive heart failure was acute myeloblastic leukemia. This study is being repeated using a lower monthly dose of adriamycin.

In addition to the Laboratory research effort, each senior investigator is involved in the teaching and training of visiting fellows, medical students and graduate students, staff fellows, clinical associates, etc. Several other investigators who are interested in learning specific techniques also spend short periods of time in our laboratory. The senior investigators are also responsible for the administration of one or more contracts with outside research institutions. The contracts are concerned with development of model tumor systems, liposomal encapsulation of antitumor agents, studies on resistance and mechanism of resistance to new antitumor agents, studies of the adverse effects of antitumor agents, and the study of the metabolism and the pharmacokinetics of new antitumor agents in laboratory animals and in humans. Agents under evaluation in these contracts are in high priority for clinical development in the Developmental Therapeutics Program and the Division of Cancer Treatment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03500-14 LCHP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Distribution of Drugs Between Blood, Brain, and Cerebrospinal Fluid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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7.0	4.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The range of brain capillary permeability measurements has been extended by use of the arterial integral method. A 5 isotope combination permits the simultaneous measurement of cerebral blood flow, vascular volume, and the permeability of brain capillaries to 3 compounds of different molecular size. A new quantitative autoradiographic technique using ^{14}C-α-aminoisobutyric acid (AIB) was developed which can measure up to a 100 fold increase in capillary permeability in focal brain regions 100 microns in diameter; tumors, X-irradiation, ischemia, and the freeze lesion were studied. The delayed effects of X-irradiation begin with focal changes in the permeability of the capillaries and progress to graded size opening of the blood-brain barrier. Cerebral edema may be generated through osmotic as well as hydrostatic pressure gradients across the capillaries; bulk flow of edema fluid from a lesion site along known anatomic pathways to specific sites remote from the lesion was supported. Low energy microwave irradiation did not alter capillary permeability or cerebral blood flow. Metrizamide was found to cross brain capillaries very slowly; metrizamide does diffuse from CSF into brain tissue but does not appreciably enter normal brain cells. Spirohydantoin mustard crosses brain capillaries and enters brain cells very rapidly.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The objectives of this project are the quantification of the transport of various materials of physiological, pharmacological, and clinical importance between blood, neural tissue and cerebrospinal fluid (CSF) and to further understand the mechanisms which regulate brain volume. The effects of various pathological conditions and pharmacological agents upon the transport and metabolism of essential physiological substances and drugs and the effects on bulk movement of fluid and water between various compartments of the blood-brain-CSF system are the focus of our investigations. The characterization of the transport properties of the blood-brain-CSF system, the study of volume regulation within the central nervous system (CNS), the development of better dose schedules and methods to obtain more effective concentrations of drugs in the CNS, and the application of these findings to improve the diagnosis and advance the treatment of patients with CNS disease are the goals of this work.

Methods Employed:

New methods have been developed to measure brain capillary permeability in both the normal and diseased brain. Capillary permeability may be expressed in terms of a unidirectional rate constant for blood to brain extracellular fluid (ECF) flux (K_i) and for brain ECF to blood flux (K_o). The methods utilize either: (1) an increasing arterial blood curve (intravenous infusion) for compounds or drugs which permeate the capillaries readily or (2) a decreasing arterial blood curve (intravenous bolus) for substances that cross the capillaries slowly. The transcapillary rate constants are calculated from the amount of the test substance in the tissue and the arterial blood curve.

Utilizing the above methods, a 5 isotope combination was developed which permits the simultaneous measurement of cerebral blood flow, vascular volume, and the permeability of brain capillaries to 3 or 4 different sized molecules. Cerebral blood flow was measured with ^{125}I-iodoantipyrine; vascular volume was determined with ^{51}Cr-RBC's or ^{111}In-transferrin (α_1-globulin); the permeability of brain capillaries to ^{24}Na-tracer sodium, ^{57}Co-diethylenetriaminepentaacetic acid (DTPA), ^{125}I-human serum albumin or ^{111}In-transferrin was measured.

A new autoradiographic technique, based in part on the above principles has been developed which can measure focal changes in brain capillary permeability. The molecule being studied is a small synthetic non-metabolized amino acid, α-aminoisobutyric acid (AIB). The technique is based on the observation that AIB flux across normal brain capillaries (blood to brain ECF) is very slow; however, once having gained access to the brain ECF, AIB is rapidly taken up and concentrated by surrounding brain cells. An increase in capillary permeability results in a corresponding increase in AIB in the brain. AIB concentration is determined from optical density measurements of the images of the tissue sections and calibrated ^{14}C-standards on the X-ray film. Utilizing a computerized densitometer and video display system, it is possible to reconstruct the autoradiograms in color (Louis Sokoloff, NIMH). Optical density can be coded to a color spectrum and the color spectrum can be programmed to serial AIB concentrations in the tissue or directly to serial AIB influx rate constants (K_i).

Ventriculocisternal perfusion with serial periventricular tissue sampling, a technique which was developed in this laboratory, is still being used. Current interest focuses on the ability to (1) obtain the brain ECF to blood efflux rate constant (K_o) for solutes which cross the capillary at a moderate rate and to (2) calculate the rate constants for solute flux into and out of brain cells (K_i' and K_o') from data obtained with this technique.

Various brain lesions are being studied using several of the above experimental techniques: (1) freeze lesion (rat), (2) X-irradiation injury (rhesus monkey), (3) metastatic and chemically induced brain tumors (rat), (4) hyperosmotic barrier opening (rat), (5) cerebral ischemia (gerbil, rat), (6) low energy microwave irradiation (rat).

Computer modeling to predict the distribution of drugs and other solutes between blood, brain, and CSF has been extended and has aided in the experimental design of several studies.

Major Findings

The arterial integral method which can measure brain capillary permeability to substances which cross the capillaries very slowly, significantly extends the range of our measurements. For comparative purposes, the arterial integral method is 1000 times more sensitive than the single capillary passage technique of Oldendorf: Brain Uptake Index (BUI). It is about 10 times more sensitive than other methods which maintain a constant blood level of test substance. Furthermore, it may be possible to determine both the influx and the efflux rate constants across the capillary and calculate the brain to blood partition coefficient under certain conditions. Recently measured blood to brain ECF (influx) rate constants (K_i) for ^{111}In-transferrin, ^{57}Co-DTPA, and ^{24}Na-tracer sodium were 6×10^{-5}, 4×10^{-5}, and 7×10^{-4} min^{-1}, respectively, in the normal rhesus monkey. These rate constants were 3 to 5 times higher in the normal rat.

A major impetus to develop the arterial integral method was due to problems associated with the BUI technique of Oldendorf. Critical evaluation of this single capillary passage technique in our laboratory and elsewhere has demonstrated that the BUI is dependent on many factors such as cerebral blood flow, sampling time, intracarotid injection volume, choice of reference tracer as well as test substance, etc. The BUI is an "index" of all these interrelated parameters and is not just an index of the permeability of brain capillaries to the test substance. Furthermore, the choice of test substances which can be studied by the Oldendorf technique is limited. The test substance must permeate brain capillaries fairly rapidly; otherwise, a very low or "background" BUI will be measured. The lower limits of blood to brain flux, expressed as a transcapillary rate constant (K_i), that can be measured by the single capillary passage technique of Oldendorf is approximately 2×10^{-2} min^{-1}. Since we are frequently interested in drugs and compounds whose transport across brain capillaries is comparatively slow ($K_i < 10^{-2}$ min^{-1}), other methods to measure the permeability of brain capillaries to these substances must be used.

The autoradiographic technqiue, using ^{14}C-α-aminoisobutyric acid (AIB), can measure a large change in brain capillary permeability in very small regions of the brain. It is possible to quantify up to a 100 fold increase and estimate up to a 1000 fold increase in the permeability of brain capillaries to AIB on the autoradiographs. It is possible to localize an increase in capillary permeability to brain regions as small as 100 to 200 microns in diameter on the autoradiographs. Color reconstructions of the autoradiographs enhances the appreciation of differences in AIB transport between neighboring brain regions and simplifies the quantitative analysis. The blood to brain ECF (influx) rate constant for AIB is 5×10^{-4} and 2×10^{-3} min^{-1} in the normal rhesus monkey and rat, respectively.

Autoradiographs of several brain lesions using ^{14}C-AIB have been prepared; they include the freeze lesion, X-irradiation injury, metastatic and chemically induced brain tumors, hyperosmotic blood-brain barrier opening, cerebral ischemia, and low energy microwave irradiation. Preliminary studies with the metastatic brain tumor model indicate that: (1) central tumor regions generally have very permeable capillaries, (2) some peripheral tumor regions demonstrate only small increases in capillary permeability, (3) in some regions where a sharp boundary between tumor and "normal brain" appears to exist on histological staining, an abrupt change in capillary permeability is seen. Preliminary studies with the X-irradiation injury model indicate that the first changes in capillary permeability may occur in cortex rather than white matter and these may preceed any histological evidence of capillary disruption or cerebral edema. The freeze lesion is sharply demarcated; there does not appear to be more than 1 to 2 mm between totally disrupted capillaries and "normal" capillaries. A two hour exposure to low energy microwave irradiation did not increase the permeability of brain capillaries to AIB.

The effect of 3500 rads of orthovoltage radiation, single dose, to the right occipital hemisphere of the rhesus monkey causes a predictable brain lesion after a 4 to 5 month delay (William Caveness, NINDS). We studied the magnitude of blood-brain barrier disruption at the site of irradiation and at various sites remote from the lesion using a 5 isotope combination (Methods, II). This data was compared with the water content of the individual tissue samples. A correlation between increased capillary permeability and cerebral edema at the lesion site and in the adjacent white matter was demonstrated. A graded size or pore opening of the barrier appears to exist; the increase in permeability of the capillaries was consistantly greater for tracer sodium than for DTPA; the permeability increase was least with respect to transferrin. In contrast, several brain regions remote from the irradiation lesion were edematous but did not demonstrate a significant increase in capillary permeability. This observation is consistant with the bulk movement of edema fluid from the lesion along known anatomic pathways to specific sites remote from the irradiated tissue. Cerebral blood flow was reduced by more than 50 percent in the lesion and adjacent edematous white matter.

The distribution of spirohydantoin mustard (SHM) in the brain and systemic organs during intravenous infusion was studied in rats. Dimethylsulfoxide (DMSO), the vehicle used to keep SHM in solution, induced systemic hypotension and a fall in cerebral blood flow. Cerebral blood flow was measured simultaneously in these experiments using ^{125}I-iodoantipyrine. The rate constants for SHM exchange across the capillaries could not be accurately measured due to the progressive fall in blood pressure and cerebral blood flow during the experiments. However, a comparison with iodoantipyrine indicates that SHM crosses brain capillaries rapidly and that its brain to blood partition coefficient is in the range of 1.

Iodoantipyrine permeates brain capillaries very rapidly and is used to measure cerebral blood flow; therefore, it is an ideal reference compound to be included in studies where the test drug or compound crosses the capillaries readily. The exchange of iodoantipyrine across the capillaries (blood to brain and vice versa) was studied and the brain to blood partition coefficient was determined.

The time course of high dose methotrexate (MTX) and citrovorum factor (CF) distribution--alone and in combination--to brain, CSF and various organs after intravenous injection is being studied in the cynomolgous monkey. MTX, CF, and 5-methyltetrahydrofolate (5-MTHFA) concentration is being determined by microbiological assay. Included in the intravenous injection are tracer quantities of ^{24}Na-sodium, ^{57}Co-DTPA, and ^{125}I-human serum albumin to further characterize blood brain barrier function in the presence of high dose MTX therapy. The time course of MTX, CF and 5-MTHFA activity in plasma, brain, CSF and various organs as well as the calculation of the rate constants for brain capillary and cell exchange should be forth coming.

The laboratory has a continuing interest in the complex system which regulates brain volume and in the alteraction this system undergoes when the brain swells and cerebral edema develops. This was outlined in last year's report. Cerebral edema is a major complication of both primary and metastatic brain tumors and often leads to clinical deterioration far in excess of that related to the tumor mass itself. Although the edema associated with brain tumors is considered to be "vasogenic", edema formation and resolution around tumors is probably more complex. We are studying several models of brain edema in an attempt to better understand some of the principles involved; the freeze lesion is being used as a model of vasogenic edema and cerebral ischemia is being studied as a possible model of osmotic edema. Published observations and preliminary experimental results, computer modeling, and theoretical considerations indicate that: (1) fluid movement from blood to brain occurs across disrupted capillaries which are permeable to protein; the driving force for fluid movement is the hydrostatic pressure gradient between the blood and brain tissue compartments. (2) Water movement from blood to brain ECF and from brain ECF to brain cells may also occur and produce cerebral edema. A specific set of conditions must exist across the capillary or cell membrane for a water (volume) shift to occur. In either case, an effective osmotic pressure must develop across the capillary and/or cell membrane to drive the movement of water from one compartment to another. For an osmotic pressure to develop across a membrane, the osmotically active solutes cannot freely exchange across that membrane. (3) The rate of fluid or water movement across the capillary or cell wall is dependent on the hydraulic conductivity of the membrane as well as the hydrostatic and osmotic pressure gradients. (4) The flow of edema fluid through brain ECF is a function of the hydrostatic pressure gradient through the ECF and the hydraulic conductivity of the extracellular space (ECS). The hydraulic conductivity is the reciprocal of resistance to bulk fluid flow and depends on several factors; one of the most important factors is the cross-sectional area of the ECS channels through which the edema fluid is flowing.

The blood-brain-CSF distribution of metrizamide during intravenous infusion and ventriculocisternal perfusion (VC-P) was studied in rabbits. Blood to brain transfer of metrizamide is very slow. Metrizamide exchanged with a 1 to 2 percent brain space after 2 hours and a 2 to 3 percent brain space after 4 hours; this corresponds to a transcapillary rate constant (K_i) of approximately 1×10^{-4} min^{-1}. Increasing the concentration of metrizamide in the plasma had no effect on the rate of blood to brain transfer. VC-P studies demonstrated that metrizamide diffused across the ependyma from CSF into and through the brain ECF; the periventricular concentration profiles of metrizamide were very similar to those of the extracellular compound ethylenediaminetetraacetic acid (EDTA) which was included in the perfusate and indicates that metrizamide does not appreciably enter normal brain cells.

Low energy microwave irradiation of the rat, frequency 2450 MHz, power density 10 MW/cm^2, did not increase the permeability of brain capillaries to tracer sodium, DTPA, or transferrin and cerebal blood flow was not altered.

Significance to Biomedical Research and the Program of the Institute

A basic understanding of the movement of drugs, physiological substances, tracer compounds, and fluid between blood, brain, and CSF is essential to appreciate both normal and abnormal central nervous system function. Specific contributions relate to the following: (1) The arterial integral methods extends the range of capillary permeability measurements; it can be applied to the study of drugs and substances which were considered not to permeate brain capillaries. The use of a 5 isotope combination allows the simultaneous measurement of capillary permeability of several different substances of different molecular size as well as the measurement of cerebral blood flow and vascular volume. (2) The autoradiographic technique using AIB can study focal permeability changes in various disease states. AIB may also have direct clinical application; it probably is 10 to 100 times more sensitive than DTPA, RISA, or meglumine diatrozate and like compounds for detecting blood brain barrier defects. Positron emitting isotopes of AIB could be used for brain scanning and computerized emission tomography. If iodinated analogues are shown to retain the transport characteristics of AIB, they should be tested as contrast enhancing agents for computerized absorption tomography. (3) Quantitative autoradiography with the appropriate ^{14}C-labeled drugs can measure the transcapillary rate constant in focal brain regions and map the distribution of drug and/or drug metabolite in the brain. Similarly, drug uptake and distribution in various in vivo brain tumor models could be studied and could help resolve the questions concerning the delivery of lipid insoluble drugs - drugs which permeate normal brain capillaries very slowly-to the tumor center and to the rapidly proliferating tumor edge. The autoradiographic observations in animal models could be extended to the study of drug distribution in patients with brain tumors using the appropriate positron emitting isotopically labeled drug and computerized emission tomography. With the ability to quantify the drug activity from the tomograms, it should be possible to individualize dose scheduling for each patient. (4) Basic information of methotrexate (MTX) and citrovorum factor (CF) entry and accumulation in brain, CSF, and various organs over time is relevant to current clinical use of high dose MTX therapy for the treatment of systemic tumors and metastasis to the brain. Computer modeling using the experimentally determined rate constants for the transcapillary and cell exchange of drugs could help establish optimum dosage schedules in patients. Several other drugs, including spirohydantoin mustard (SHM), are being studied. (5) Orthovoltage irradiation of the brain remains one of the major therapeutic options currently available for the treatment of both primary and secondary brain tumors. Continued research efforts directed to more effective modes of treatment and to an understanding of the toxicities associated with that treatment are warranted. Our efforts are directed toward the sequence of events associated with small changes in the permeability of brain capillaries and the progression of the injury leading up to the necrosis of brain tissue, massive opening of the barrier, and the brain swelling that accompanies the full blown irradiation lesion. (6) The edema associated with several brain lesions, including tumors, remains a major clinical problem. Often the edema associated with a lesion results in clinical deterioration far in excess of that related to the size of the lesion itself. There are several mechanisms for fluid movement from blood to brain. Osmotic pressure gradients may be as important as hydrostatic pressure gradients, particularly if the capillary and/or cell reflection coefficients of sodium and chloride are significantly reduced. A better understanding of the generation and resolution of osmotic pressure gradients may lead to more effective therapy of cerebral edema. (7) Diagnostic studies with intrathecal metrizamide should consider that the largest fraction of the drug's clearance from the CSF occurs through the pathways of bulk CSF absorption and therefore is directly related to CSF turnover. In disease states where CSF turnover decreases, i.e., hydrocephalus or partial blockage of a CSF compartment, a greater fraction of metrizamide will diffuse into the brain parenchyma and may be associated with increased neurotoxicity. Metrizamide is cleared very slowly across normal brain capillaries.

Proposed Course:

The Membrane Transport Section plans to continue and follow up the basic research goals outlined above and to initiate collaborative studies to confirm the clinical application of several of the observations we have made in the laboratory. Specifically, efforts will be focused on the following: (1) Extend the application of quantitative autoradiography to other tracer molecules (EDTA, albumin, etc.) and to selected drugs in the study of brain tumors and other lesions. (2) Set up a quantitative double-label autoradiographic method to measure changes in capillary permeability with respect to two substances of different molecular size simultaneously. Quantitative double-label autoradiography will also be useful in the study of drug distribution in tumor or abnormal tissue because a reference compound can be included. (3) Study the capillary and brain cell transport characteristics of iodinated analogues of AIB and/or other small neutral amino acids in hope of finding a new class of contrast enhancing compounds which will improve the detection of blood-brain barrier defects by computerized absorption tomography. (4) Attempt to implement collaborative studies which could confirm the clinical application of AIB to detect subtle disruptions of the blood-brain barrier by brain scanning and by computerized emission tomography. (5) Apply our current methods to measure the transcapillary rate constants and brain to blood partition coefficient of selected drugs with current clinical interest. Two drugs planned for study are N-(Phosphonacetyl)-L-aspartate, PALA (NSC-224131) and 1,4-cyclohexadiene-1,4-dicarbamic acid, 2,5-bis(1-aziridinyl)-3,6-dioxodiethylester, AZQ (NSC-182986). (6) Continue our ongoing studies in cerebral edema and brain volume regulation. Further studies with the "vasogenic model" of cerebral edema using the above methods are planned. We also hope to develop a model of "osmotic edema"; studies will focus on the permeability of the capillaries in the edematous tissue, the osmotically effective solutes (proteins, polypeptides, lactic acid, etc.), and the resolution of the edema. (7) Continue our transport studies in normal animals with physiological substances--such as ions, amino acids, sugars, and proteins--to further characterize the flow of solutes and water between blood, brain, and CSF. (8) Discontinue studies using the intracarotid injection-single capillary passage technique of Oldendorf.

Publications:

1. Fenstermacher, J. D., and Cowles, A. L.: Theoretic limitations of intracarotid infusions in brain tumor chemotherapy. Cancer Treat Rep. 61: 519-526, 1977.

2. Blasberg, R. G.: Methotrexate, cytosine arabinoside, and BCNU concentration in brain after ventriculocisternal perfusion. Cancer Treat Rep. 61: 625-631, 1977.

3. Blasberg, R. G., Patlak, C. S. and Shapiro, W. R.: Distribution of methotrexate in the cerebrospinal fluid and brain after intraventricular administration. Cancer Treat Rep. 61: 633-641, 1977.

4. Blasberg, R. G.: Pharmacodynamics and the Blood-Brain Barrier. Symp. on Modern Concepts in Brain Tumor Therapy, Natl Cancer Inst. Mong. 46: 19-27, 1977.

5. Cserr, H. F., Fenstermacher, J. D., and Rall, D. P.: Comparative aspects of brain barrier systems for nonelectrolytes. Am. J. Physiol. 234: R52-R60, 1978.

6. Editors: Bito, L., Davson, H., and Fenstermacher, J. The Ocular and Cerebrospinal Fluids. London, Academic Press, p. 561, 1977.

7. Blasberg, R. G.: An outline of problems associated with quantifying the effects of microwave irradiation on blood-brain barrier function. Radio Science 13/65, 1978 (in press).

8. Caveness, W. F., Kemper, F. L., Brightman, M. W., Blasberg, R. G., and Fenstermacher, J. D.: The directional character of vasogenic edema. Proceedings of the International Erwin Reich Symposium on the Pathology of Cerebrospinal Microcirculation, 1978 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03502-15 LCHP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Folic Acid Antagonists in Experimental Tumors and Normal Tissues of Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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SECTION
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INSTITUTE AND LOCATION
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TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.25	OTHER: 0.45

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The pharmacokinetics of methotrexate in Lewis Lung tumor bearing mice is being studied. The K_m and V_{max} for in vivo transport in this tumor has been quantitated.

The metabolites of methotrexate present in the plasma of mice are being investigated by HPLC techniques.

Quantitation of nucleosides and citrovorum factor needed to reverse methotrexate toxicity was investigated.

[illegible]-6040
(Rev. 10-76)

Project Description

Objectives:

1. To develop and refine a pharmacokinetic model for MTX.

2. To study the biochemical and toxic effects of MTX and reversal of these effects with citrovorum factor and various nucleosides.

3. To attempt to relate pharmacokinetic findings to biochemical and toxic effects.

Methods and Major Findings:

Studies on MTX transport *in vivo* in Lewis Lung tumor bearing mice are being continued. We have shown previously that the enzyme dihydrofolate reductase has a V_{max} in Lewis Lung tumor six times that in small intestine and that the growth fraction in Lewis Lung tumor is about one quarter that in small intestine. Plasma and tumor MTX concentrations, after correction for MTX metabolites, were determined as functions of time over a five log range of doses (0.003 mg/kg to 300 mg/kg). In conjunction with our overall pharmacokinetic model of MTX, we determined the maximum transport rate (k) and the Michaelis constant (K) for the *in vivo* Lewis Lung tumor, (two weeks after transplantation). The ratio k/K of 0.012 min^{-1} is about one-third of the value previously obtained for small intestine, and provides additional information as to the resistance of the Lewis Lung tumor to MTX treatment.

Studies on the metabolism of MTX in mice are continuing, and methods are being developed to isolate the major metabolites and identify them by field desorption mass spectrometry.

Past studies have shown that plasma MTX concentrations of 2×10^{-8} M, maintained by constant infusion for prolonged periods (∿ 100 hours), are lethal to mice. Concomitant biochemical tracer studies indicated that whereas incorporation of ^{3}H-UdR into DNA of small intestine was almost completely inhibited, ^{3}H-TdR incorporation was stimulated. This finding suggested that TdR alone might reverse the toxicity of 2×10^{-8} M MTX, and that purines were not limiting. Earlier *in vitro* bone marrow cloning studies indicated that the TdR concentration needed to reverse toxicity of any MTX concentration from 10^{-8} to 10^{-4} M was optimal at 10^{-5} M but required the presence of purines. More recently, we have found that the toxicity of a prolonged plasma MTX concentration of 2×10^{-8} M is reduced only slightly by a TdR infusion which results in plasma TdR concentrations of 2×10^{-6} M, but is completely reversed when plasma TdR concentrations are maintained at 2×10^{-5} M. However, a plasma TdR concentration of 2×10^{-5} M does not afford complete protection against the toxicity of higher plasma MTX concentrations (2×10^{-7} M) at which the incorporation of both ^{3}H-UdR and ^{3}H-TdR into DNA are inhibited. Our previous studies using bone marrow cells showed that concentrations of CF equal to, or greater than, MTX concentrations were required to reverse MTX-induced inhibition of clone formation. More recent results indicate that the *in vivo* lethality of MTX in mice is reversed by CF at concentrations one tenth those of MTX. Whereas, if the degree of reversal of inhibition of ^{3}H-UdR incorporation is taken as a measurement of toxicity, similar conclusions as arrived at from *in vitro* cloning studies are found, that is, that equal or greater ratios of CF:MTX are needed to reverse toxicity. This discrepancy is as yet unexplained.

Publications

1. Zaharko, D. S.: Chemokinetics. *Environ. Health Perspect.*, Feb. 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03505-15 LCHP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Potential Embryotoxic, Carcinogenic and other Adverse Effects of Anticancer Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

New antitumor agents, as well as clinically established drugs are being evaluated for embryotoxic, cytogenetic and carcinogenic effects. A comparative study of the embryotoxic and cytogenetic effects in mice of a series of plant-derived antitumor agents (vincristine, maytansine, colchicine, VP 16-213 and VM-26) has been completed, as have similar studies on two adenosine deaminase inhibitors (2'-deoxycoformycin and EHNA). Studies have been initiated on the embryotoxic and cytogenetic effects of two inhibitors of de novo pyridmide nucleotide biosynthesis (N-[phosphonacetyl]-L-aspartic acid and 6-azauridine) and several anthracenediones. The carcinogenic activity of various clinically effective antitumor and/or immunosuppressive agents, including procarbazine, N-methylnitrosourea, melphalan, azathioprine and adriamycin, is under evaluation in long-term studies in monkeys. A survey of second malignancies developing in treated cancer patients, initiated several years ago, is continuously being updated using data from published reports and from several centers and hospitals.

Project Description;

Objectives:

This project is designed to obtain data on various aspects of the toxicity of antitumor agents, including their embryotoxic, cytogenetic and carcinogenic effects in rodents, monkeys and in the human. Specifically, the objectives are:

To evaluate in mice the embryotoxic and cytogenetic effects of various classes of antineoplastic agents. These agents include selected plant-derived drugs with clinical as well as experimental antitumor activity, adenosine deaminase inhibitors, inhibitors of de novo pyrimidine nucleotide biosynthesis and several anthracenediones. The goal of such studies is to define differences in teratogenic potency among groups of agents with similar mechanisms of antitumor action and antitumor spectra. In addition, the studies are designed to provide information as to the period of embryonic development most sensitive to the effects of the test compounds. The possibility of preventing antitumor agent-induced embryotoxicity with various chemicals is being evaluated. Studies are also underway to follow the time course of embryonic drug-induced cytogenetic damage, the extent to which embryonic tissue is capable of repairing such damage, and the relationship of embryonic cytogenetic damage to developmental abnormalities.

To evaluate in monkeys the carcinogenic potential of various clinically useful antitumor and/or immunosuppressive agents. In addition, the effects of such treatment on other functions, such as the immune system and reproduction, is under examination. A signifiant proportion of monkeys receiving long-term treatment with procarbazine has developed a malignancy, about half of which have been acute leukemia (see below). The surviving monkeys presently receiving procarbazine are considered to be at high risk of developing acute leukemia, and hematologic and cytogenetic studies are currently being carried out in this group of animals. The goal of these studies in the procarbazine-treated monkeys is to determine whether a "preleukemic" condition exists in chemically-induced acute leukemia, and if so, to characterize it.

To monitor the current medical literature and the NCI patient data in order to tabulate and characterize adverse effects of antineoplastic agents in man. Particular emphasis is being given to embryotoxic, mutagenic and carcinogenic effects arising from treatment with clinically effective antitumor agents.

Major Findings:

Embryotoxicity of Antitumor Agents

The teratogenic effects of 3 new plant-derived antitumor agents, maytansine, VP-16-213 and VM-26, were compared to the effects of vincristine and colchicine in pregnant Swiss albino mice that received a single ip injection of drug on day 6,7 or 8 of gestation. Cytogenetic studies were also performed using maternal bone marrow and embryos obtained 48 hours after injection of maytansine, vincristine, VP-16-213, VM-26 and colchicine on day 6, 7 or 8 of gestation. A close correlation between teratogenic and cytogenetic effects was not noted among the compounds tested. Vincristine had greater embryotoxic and teratogenic activity than maytansine at equimolar doses (0.36 μmoles/kg), with the peak effects appearing after injection on day 7 of gestation. Colchicine, VP-16-213 and VM-26 were comparatively less potent than maytansine and vincristine, since doses of 2.5 μmoles/kg (colchicine and VP-61-213) and 1.5 μmoles/kg (VM-26) were required to elicit embryotoxic effects. At their teratogenic doses, all compounds induced various cranial abnormalities, including exencephaly, hydrocephalus, anophthalmia and microtia, as well as major skeletal malformations. The teratogenic dose of vincristine is comparable to its effective antitumor dose in transplantable rodent tumor systems; in contrast, the teratogenic dose of maytansine is approximtely 10-fold higher than its antitumor dose. Of the compounds studied, VP-16-213 and VM-26 exerted the most consistent cytogenetic effects in embryonic tissue. A large proportion of the structural chromosome aberrations induced in embryonic cells by VM-26 were stable and are most likely capable of surviving at least one cell division.

Studies on the embryotoxic and cytogenetic effects of 2 inhibitors of the enzyme adenosine deaminase, 2'-deoxycoformycin (DCF) and erythro-9-(2-hydroxyl-3-nonyl)-adenine (EHNA), have been completed. DCF administered daily on days 7-11 of gestation did not possess marked embryotoxic activity at the dose (0.25 mg/kg) found optimal for enhancement of the antitumor activity of adenosine analogs against experimental animal tumors. Although a dose-related increase in the incidence of intrauterine mortality was noted at higher (0.375-2.0 mg/kg) dose levels of this drug, a statistically significant effect on the body weights or on the incidence of congenital malformations in the surviving fetuses was not found. Similarly, when single injections of DCF were given on day 6,7,8 or 10 of gestation, a statistically significant embryolethal, but not teratogenic, effect was observed only at the highest dose (2.0 mg/kg) tested. Results with EHNA indicate that it also is without striking embryotoxic activity when given on days 7-11 of gestation at doses (25,50 and 100 mg/kg) known to potentiate the antitumor action of adenosine analogs. When given as a single dose on day 6, 7 or 8 of gestation, EHNA induced statistically significant increases in the incidence of intrauterine mortality and fetal malformations on day 8 of gestation only at the highest doses (150 and 200 mg/kg) tested. However, it should be noted that these doses were hypnotic in pregnant mice, since the mice slept for 1-2 hours following treatment.

Studies are in progress on the embryotoxicity of PALA and 6-azauridine, two inhibitors of de novo pyrimidine nucleotide biosynthesis. Daily treatment with PALA for 5 days during early gestation resulted in 100% intrauterine deaths in litters exposed to a dose (3 mg/kg/day) approximately 100-fold lower than that found optimal for increasing the survival of mice inoculated with B16 melanoma and Lewis lung carcinoma. Approximately 50% of fetuses survived daily PALA doses of 1.5 mg/kg, and a dose of 0.75 mg/kg was without embryolethal effects. The effects of single doses PALA given on day 4,5,6, 7,8,9 or 10 of gestation have also been evaluated. The peak of sensitivity to the embryolethal effects of PALA occurred on days 7 and 8 of gestation, when a dose of 10 mg/kg induced significant embryolethality and a 25 mg/kg dose induced 100% intrauterine deaths. When given on day 9 of gestation, PALA at 12.5 mg/kg was embryolethal, although even a 25 mg/kg dose was not 100% effective in inducing intrauterine deaths. The mouse fetus at 10 days gestation was relatively insensitive to the embryolethal effects of PALA, since approximately 20% survived exposure to 200 mg/kg. Fetuses exposed to PALA at early gestational stages are also relatively resistant to the embryolethal effects of this drug. When given on day 4,5, or 6 of gestation, significant embryolethality was found only at 100, 50, and 25 mg/kg respectively, and no embryos survived exposure to 200 mg/kg. PALA exerted teratogenic effects in the fetuses surviving doses of the drug ranging from 5-200 mg/kg given after day 5 of gestation. The peak of sensitivity to PALA-induced teratogenicity occurred on day 7. Thirty-five percent of live fetuses exposed to PALA on day 7 of gestation were malformed, whereas when this dose was given on day 6 or 8 of gestation, only 3.4% and 5.6%, respectively, of fetuses were malformed. A high rate (70%) of malformations, exclusively of the skeleton, was also noted in fetuses surviving a dose of 200 mg/kg given on day 10 of gestation. The possibility of reversing the embryolethality of PALA by uridine is currently being evaluated. In order to optimize the schedule of uridine doses, the persistence of ^{14}C-activity in maternal plasma and its uptake by the embryo was measured at intervals after an oral dose of (2-^{14}C)-uridine to pregnant mice. The peak of radioactivity in both maternal plasma and embryonic tissue occurred 30-45 minutes after dosing; ^{14}C-activity declined rapidly after 60 minutes, but was still detectable 3 hours after dosing. In the reversal studies, 8-day pregnant mice were given an oral dose of uridine 30 minutes prior to a 100% embryolethal ip dose of PALA. Additional doses of uridine were given 30 minutes (7 doses), 45 minutes (7 doses), 60 minutes (5 doses) or 120 minutes (3 doses) after PALA treatment. The best schedule tested to date involves 7 doses of uridine spaced 45 minutes apart, which decreased the embryolethality of PALA (25 mg/kg) from 100% to 65%. However, preliminary results suggest that a large proportion of the surviving fetuses are malformed. Efforts to improve the reversal of PALA-induced embryotoxicity with uridine are continuing. In addition, (acetyl-^{14}C)-PALA has recently become available, and studies on its physiologic disposition in pregnant and non-pregnant mice are underway.

Preliminary results with 6-azauridine suggest that it too has potent embryotoxic activity. When given on days 7-11 of gestation at 37 mg/kg, a 90% incidence of intrauterine death resulted, and all of the surviving fetuses were malformed. When given at 25 mg/kg on days 7-11 of gestation, a 20% incidence of intrauterine death resulted, and 25% of the surviving fetuses were severely malformed. Single doses of 6-azauridine, at 25, 50 and 100 mg/kg, were given on day 6,7 or 8 of gestation. Although the 6-day mouse fetus is relatively insensitive to 6-azauridine, on both day 7 and day 8 of gestation the drug induced dose-related increases in the incidence of intrauterine deaths and fetal malformations. Only one of 77 fetuses exposed to 6-azauridine on day 7 of gestation survived, and this fetus had numerous severe malformations. Given on day 8 of gestation, 6-azauridine induced a 78% incidence of intrauterine deaths, and 60% of the surviving fetuses were malformed.

Studies on the embryotoxicity of two anthracenediones have recently been initiated. Dihydroxyanthracenedione (NSC-279836) has been given to pregnant mice on day 7, 8 or 9 of gestation at doses of 1, 5 or 10 mg/kg. Although none of the doses tested has produced a significant increase in the incidence of intrauterine deaths on day 7, 8 or 9 of gestation, a dose of 10 mg/kg given either on day 7 or 8 of gestation has resulted in a fetal malformation rate of 39% and 46%, respectively. The parent compound, anthracenedione (NSC-287513) has to date only been tested in 9-day pregnant mice at doses of 5, 25 and 50 mg/kg. Under these experimental conditions, the compound does not appear to possess marked embryotoxicity.

Evaluation of antitumor agents for carcinogenic activity in monkeys

From our survey of second tumors in treated cancer patients, we are accumulating evidence that Hodgkin's disease patients receiving treatment with the MOPP regimen, one component of which is procarbazine, are at increased risk of developing AML. Although a causal relationship between cytotoxic drug therapy and the appearance of AML in these patients has not yet been established, there is ample evidence that procarbazine is a potent carcinogen in mice and rats. In addition, the carcinogenic potential of procarbazine in 3 species of non-human primates is being evaluated. A total of 55 monkeys have received procarbazine for periods up to 11 years. Eleven of the 43 monkeys (26%) necropsied thus far have had malignant neoplasms, six of which were acute leukemia. Two monkeys developed osteogenic sarcomas, 2 monkeys developed hemangiosarcomas, and a single case of lymphocytic lymphoma was found. The average total dose of procarbazine received by the monkeys developing malignancies was 36.0 gm, the average duration of procarbazine treatment was 75 months. The 12 surviving monkeys in this study may represent a population at high risk for developing AML. For this reason, they are being utilized in hematologic and cytogenetic studies in order to determine whether a "pre-leukemic" phase is detectable prior to the development of frank drug-induced leukemia. For this purpose, sequential bone marrow samples from all of the monkeys receiving procarbazine are being examined for alterations in cellular morphology and for chromosomal aberrations. Since initiation of these studies, no specific chromosomal aberrations have been detected in bone marrow cells from the monkeys and no additional cases of acute leukemia have developed. However, a number of the toxic effects of procarbazine seen clinically are also noted in the monkeys, including vomiting and myelosuppression. Its most striking toxic effect, however, is on the reproductive system of the males. The majority of the adult males necropsied to date have had testicular atrophy with complete aplasia of the germinal epithelium.

There is some evidence that patients receiving long-term treatment with melphalan for multiple myeloma or ovarian cancer may also be at increased risk of developing AML. Although a causal relationship between melphalan treatment and the development of AML in these patients has not yet been established, the carcinogenic activity of this agent has been demonstrated in mice and rats. The carcinogenic potential of melphalan in non-human primates is therefore under study. Twenty monkeys are currently being treated by the oral route with melphalan (0.1 mg/kg) daily, 5 days a week. To date, none of these monkeys have developed a malignancy; however, this study has been underway for less than 3 years.

Kidney transplant recipients and other patients under chronic immunosuppressive therapy with azathioprine appear to be at risk of developing malignancies, primarily lymphomas. Whether this increased risk is related to a direct oncogenic effect of azathioprine or is secondary to a prolonged immunosuppressed state is at present unclear. The carcinogenic potential of azathioprine is being evaluated in a group of 15 monkeys receiving oral doses of the drug daily, 5 days a week. This study has been in progress for 16 months and none of the monkeys has yet developed a malignancy.

N-methylnitrosourea (MNU) is under clinical trial in the USSR and has been reported to be effective in the treatment of lung cancer. We have evaluated its carcinogenic potential in three species of non-human primates. A total of 43 monkeys have received oral doses of MNU for periods up to 124 months. Six of the 18 monkeys (33%) necropsied thus far have had squamous cell carcinoma (SCA) of the mouth, pharynx and/or esophagus, and upper digestive tract lesions such as atrophy or dyskeratosis of the esophageal mucosa and esophagitis have been a consistent finding among the 18 monkeys necropsied to date. All but one of the animals receiving total doses of MNU exceeding 50 gm developed SCA after an average of 6 years of exposure. Many parallels were noted between the esophageal SCA observed in the present series of monkeys and human esophageal carcinoma, including the clinical manifestations of the tumor, its complications, its radiographic appearance and its morphology. The MNU-induced lesions of the oro-pharynx and esophagus of primates may therefore be a valuable model for the study of human esophageal carcinoma.

The potential carcinogenicity of adriamycin is also being evaluated. A group of 10 monkeys received an iv dose of drug (1 mg/kg) once each month for 23-28 months. At the end of the dosing period it was intended to hold the animals under observation for the remainder of their lives. However, approximately 2 months after the last dose of adriamycin 9 of 10 animals developed congestive heart failure. Histopathologic examination of cardiac muscle taken at necropsy revealed lesions characteristic of adriamycin-induced cardiomyopathy in humans, and in some cases these findings were confirmed by electron microscopy. In man, a cumulative adriamycin dose of 550 mg/m^2 has been associated with cardiac toxicity; non-human primates appear to be more sensitive to adriamycin-induced cardiomyopathy as the monkeys in the present study had received an average cumulative dose of 310 mg/m^2 (range 276-336 mg/m^2). Moreover, an additional finding at necropsy of one of the animals dying of congestive heart failure was acute myeloblastic leukemia. This study is being repeated using a lower monthly dose of adriamycin.

Publications:

1. Adamson, R. H., Krolikowski, F. J., Correa, P., Sieber, S. M., and Dalgard, D. W.: Carcinogenicity of 1-methyl-1-nitrosourea (MNU) in non-human primates. J. Natl. Cancer Inst. 59: 415-422, 1977.

2. Sieber, S. M.: The action of antitumor agents: a double edged sword? Med. Pediat. Oncol. 3: 123-131, 1977.

3. Adamson, R. H., and Sieber, S. M.: Antineoplastic agents as potential carcinogens. In Hyatt, H. H., Watson, J. D., and Winsten, J. A. (eds.): Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, Book A, pp. 429-443, 1977.

4. Adamson, R. H., and Sieber, S. M.: Carcinogenic potential of cancer chemotherapeutic agents in man. Cancer Bull. 29: 179-183, 1977.

5. Sieber, S. M., Whang-Peng, J., Botkin, C. C., and Knutsen, T.: Teratogenic and cytogenetic effects of some plant-derived antitumor agents (vincristine, colchicine, maytansine, VP-16-213 and VM-26) in mice. Teratology, (in press), 1978.

6. Sieber, S. M., Correa, P., Dalgard, D. W., and Adamson, R. H.: Carcinogenic and other adverse effects of procarbazine in non-human primates. Cancer Res. (in press), 1978.

7. Sieber, S. M., and Adamson, R. H.: Some comments on the potential carcinogenicity of the clinically useful antitumor agents. Cancer 40: 1950-1951, 1977.

8. Sieber, S. M. and Adamson, R. H.: Chemical carcinogenesis in non-human primates and attempts at prevention. In: Proceedings of the Conference on Antiviral Mechanisms in the Control of Neoplasia, 1978, in press.

9. Adamson, R. H. and Sieber, S. M.: Chemical carcinogenesis in non-human primates. In: Proceedings of the Workshop on Non-human Primates in Cancer Research, (in press), 1978.

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PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
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NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 03506-15 LCHP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Pharmacology and Disposition of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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4	2.5	1.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pharmacologic disposition studies were initiated for two new agents in laboratory animals [PALA (NSC-224131) and misonidazole NSC-261037)]. Disposition studies in laboratory animals were continued for four agents [spirohydantoin mustard (NSC-172112), cis-dichlorodiammineplatinum (NSC-119875), AMSA (NSC-141549) and imidazopyrazole (NSC-51143)]. Clinical pharmacokinetic studies are presently being conducted in patients receiving PALA, AMSA, cis-dichlorodiammineplatinum and gallium nitrate (NSC-15200). Analytical assay techniques were developed for PALA, AMSA, misonidazole and cis-dichlorodiammineplatinum to aid in the clinical evaluation of these agents.

respectively for the hydantoin-^{14}C. The pattern of disappearance of plasma radioactivity in dogs resembled that of rats, although the differences observed between the two labeled species for the unbound and extractable ^{14}C were less pronounced. Tissue distribution studies in rats and dogs at 4 hrs showed that concentrations of both labeled forms of SHM in the cerebrum, cerebellum and medulla were equivalent and were not significantly different from the levels of total radioactivty in the plasma. Also, in the rat, concentrations of both labeled species in the brain were similar to those in the liver, but were approximately 3-fold lower than those in the kidney. In dogs, levels of the ethyl-^{14}C in the brain were 2- and 3-4-fold lower than in the kidney and liver, respectively, whereas concentrations of the hydantoin-^{14}C were 2-fold lower than in the liver and kidney. Because of the difficulty in formulating a suitable parenteral form of SHM, a preliminiary study on the tissue distribution of the ethyl-labeled SHM after oral administration was conducted in rats. Radioactivity was detected in the brain, although at 4 hrs concentrations were 6-fold lower than after iv administration. Plasma levels were 3-fold lower after oral administration than after iv administration.

Disposition of N-(Phosphonacetyl)-L-Aspartate (PALA) NSC-224131, in Patients Undergoing Phase I Clinical Evaluation.

PALA has recently entered Phase I Clinical Trials. A gas chromatographic assay for this drug was developed to obtain clinical pharmacokinetic data to aid in its clinical evaluation. Using this sensitive and specific assay, levels of PALA in serum and urine can be quantitated in the range of 0.5 μg/ml to 50 μg/ml. Analysis of samples taken from patients given doses of 100 mg/m^2 and 200 mg/m^2 indicates peak PALA serum levels of 7-19 μg/ml. In two patients from whom sufficient samples were collected, the elimination half-lives were 3.9 and 6.3 hours, respectively. Analysis of urines from two patients indicated that 22% and 38% of the administered dose was eliminated in 24 hours. These values are considerably lower than those observed in the dog, in which 60% of the administered dose was excreted in the urine after 2 hours and 75% after 12 hours. Studies presently underway in the dog using C^{14}-PALA show peak serum levels of 450 μg/ml following a dose of 120 mg/kg (2400 mg/m^2) and a 10-fold decrease in serum levels in 2 hours. Levels of PALA in the CSF of the dog reached a maximum of 2.7 μg/ml, which is a cytotoxic concentration *in vitro*. Currently, three techniques (gas chromatographic analysis, radioactive assay using C^{14}-PALA, and inhibition of aspartate transcarbamylase activity) for determining levels of PALA in dog serum and urine are being compared to determine the reliability of each method and the extent to which PALA is metabolized. Information obtained from dogs relating to PALA disposition and possible metabolism will be utilized in further clinical pharmacologic studies.

The Distribution of cis-Dichlorodiammineplatinum, NSC-119875(DDP) and Analogues of DDP in Monkey CSF

Because of several clinical reports describing possible CNS toxicity following cis-DDP infusion, studies were undertaken to assess the ability of cis-

Project Description;

Objectives:

To study the metabolic fate of experimental antitumor agents prior to clinical trial and to ascertain whether their metabolites are active in various tumor systems.

To study the pharmacology of antitumor drugs of current interest in clinical chemotherapy in order to design better therapeutic regimens.

To study the clinical pharmacology of new agents in phase 1 clinical trials.

To evaluate the usefulness of new tumor systems in experimental chemotherapy.

To design new anticancer drugs by rational approaches.

Methods Employed:

Usual pharmacologic, biochemical, analytical, and immunologic techniques.

Major Findings:

Disposition of Spirohydantoin Mustard, NSC 172112, In Rats and Dogs

Spirohydantoin mustard (SHM), NSC 172112, is an antitumor agent with activity against intracranially implanted murine ependymoblastoma. Preliminary studies with the unlabeled compound in this laboratory demonstrated that small amounts of the parent drug pass the blood-brain barrier in dogs and enter the CSF. When SHM-[hydantoin-4-^{14}C] and SHM-[2-chloroethyl-U-^{14}C] became available, these studies were extended to determine the overall disposition of SHM. After administration to rats (6.25 or 3.13 mg/kg, iv.) and dogs (1 mg/kg, iv.), the renal clearance of the hydantoin-^{14}C was more rapid (29-35% within 4 hrs.) than that of the ethyl-^{14}C (14-17% within 4 hrs). In contrast, only 18% of the administered hydantoin label appeared in rat bile within 4 hrs. compared to 33-38% of the ethyl label. Chromatographic analysis of the bile after administration of ethyl-labeled SHM revealed several components. The two major radioactive metabolites are believed to be conjugates of glutathione and/or cysteine as determined by analysis of rat bile following administration of unlabeled SHM and [^{35}S]-glutathione or [^{14}C]-cysteine. The levels of total, unbound and toluene extractable radioactivity in the plasma declined in a biphasic manner. During the initial distribution phase in rats, the toluene extractable radioactivity disappeared more rapidly from the plasma ($t_{1/2}$ = 6-9 min.) than did the unbound radioactivity ($t_{1/2}$ = 14-20 min.) and total radioactivity ($t_{1/2}$ = 18-25 min.), and only small differences between the ethyl and hydantoin-labeled species were observed. However, differences between the two labeled species were noted during the second phase; the half-life values for total, unbound and toluene extractable radioactivity being 412-471, 168 and 185 minutes, respectively, for the ethyl-^{14}C and 240-256, 93 and 32 minutes, DDP to enter the CSF. Monkeys with a surgically implanted Ommaya reservoir were used to obtain serial CSF samples from the 4th ventricle at various times after an intravenous dose of cis-DDP. Platinum concentrations were determined by atomic absorption spectroscopy. Although platinum enters the CSF, maximum concentrations were only 1-3% of the peak serum concentrations. Peak CSF levels were reached 30-40 minutes after intravenous injection and, after reaching peak values, the CSF levels declined with a $T_{1/2}$ of approximately 45 minutes. The entrance of cis-DDP into the CSF was dose dependent and doubling the infused dose doubled the maximum CSF levels. Two analogues of cis-DDP, malonato 1,2-diaminocyclohexane platinum II (NSC 224964) and sulfato 1,2 diaminocyclohexane platinum II (NSC 250427), were evaluated in the monkey system in conjunction with the ongoing program in DCT to develop new platinum derivatives. The levels of malonato-platinum found in the CSF were very similar to cis-DDP, but sulfato-platinum did not achieve detectable CSF levels. When melanato-platinum was given by prolonged intravenous infusion, CSF levels were relatively higher than when the drug was given as a short intravenous infusion. This system, coupled with the analytical technique of atomic absorption spectroscopy will be used in the assessment of other platinum derivatives. Those derivatives that do not penetrate the CSF should not possess the CNS toxicity of DDP; alternatively, those that penetrate the CNS efficiently may prove useful for treating CNS malignancies.

The Pharmacokinetics of cis-Dichlorodiammineplatinum, NSC 119875 (DDP) in Patients Undergoing Phase II Clinical Trials.

Studies on the clinical pharmacokinetics of cis-DDP given as a 1 hour infusion have been completed. Platinum concentrations in urine and blood were determined by atomic absorption spectroscopy. Plasma concentrations of platinum exhibited a biphasic curve with an early fast decline, ($T_{1/2}$ = 23 min.) followed by a slower secondary phase ($T_{1/2}$ = 67 hr). Urinary excretion data were used to calculate the plasma levels of non-protein bound platinum, and markedly different kinetics were noted for this fraction of the total plasma platinum. Protein bound cis-DDP was found to have minimal cytotoxicity against L1210 cells in vitro; thus, the concentration of active drug is directly related to levels of non-protein bound platinum. Levels of non-protein bound platinum demonstrated a biphasic curve, with a rapid decline having a $T_{1/2}$ of 8-10 minutes and a slower second decline with a $T_{1/2}$ of 45 minutes. Although the total plasma platinum concentration remains high for several days after cis-DDP infusion, the non-protein bound platinum falls to low levels within several hours of infusion. The urinary excretion rate parallels the non-protein bound platinum levels and most of the urinary excretion occurs in the first hours after infusion. Therefore, the danger of acute renal damage is probably significant only during the phase of rapid excretion, and, in contrast to methotrexate, renal failure developing days after infusion would not be expected to lead to cis-DDP toxicity.

Disposition of 4'-(9-Acridinylamino)-Methanesulfon-m-anisidide (AMSA), NSC-141549 in Rats and Mice.

Previous studies on the disposition of C^{14}-AMSA (see previous annual report) were performed with the radioactive label in the 9-carbon of the acridine ring. Recently, C^{14}-AMSA was synthesized with the radioactive label in the side chain (the 4-amino-3-methoxymethanesulfonanidide moiety) and a limited disposition study was performed in mice and rats with this material. In vitro and in vivo results confirmed our previous observations that the 9-carbon of AMSA is extremely vulnerable to nucleophilic attack by alkythiols and hydroxide ion. Plasma levels in rats of unchanged C^{14}-AMSA using the labeled side group correlated with values obtained with C^{14}-AMSA labeled in the acridine ring. The liberated side chain is excreted in the bile at concentrations and rates similar to that of the metabolite containing the acridine nucleus. TLC analysis of the bile indicated the presence of the side chain plus several unidentified metabolites of the side chain not associated with the acridine moiety. Previous attempts to identify the major biliary metabolite containing the acridine moiety by conventional mass spectroscopic techniques were not successful because of the instability of the metabolite. However, using the technique of field desorption mass spectroscopy we have been able to identify the major biliary metabolite as the glutathione thioether of acridine.

Disposition Studies of 4'-(9-Acridinylamino)-methanesulfon-m-anisidide (AMSA), NSC-141549 in Patients Undergoing Phase I Clinical Trials

As part of the trials now underway to define the human toxicity and human antitumor spectrum of AMSA, efforts were made to obtain clinical pharmacokinetic data. Part of that effort consisted in the development of a fluorescence assay for the drug in serum or plasma. Under alkaline conditions, AMSA decomposes into 9-(10H) acridone which is very fluorescent and the fluorescence is used to quantitate the amount of AMSA present. The assay permits the measurement of AMSA levels to 5 x 10^{-8} M AMSA with an absolute limit of sensitivity of about 1 x 10^{-8} M AMSA. Using the fluorescence assay, a number of patients have been studied and preliminary information on drug plasma levels has been obtained. Doses of 10 mg/m^2 gave peak plasma levels of 0.7 μM, 30 mg/m^2 gave 2.0 μM, 50 mg/m^2 gave 8-9 μM, and 70 mg/m^2 gave 15-20 μM. The time course of plasma AMSA concentration shows a biphasic pattern, having a rapid α-phase decline with a half-life of approximately 30 minutes and a plateau phase with a much longer half-life. Radiolabeled AMSA has been prepared for use in human pharmacokinetic studies which will provide more detailed information on drug metabolism and excretion.

Disposition of Misonidazole, NSC-261037, in Monkeys

Phase I clinical trials were recently completed with oral administration of enteric coated tablets of the radiosensitizer drug Misonidazole (RO-07-0582). An intravenous formulation would be useful since it would: a) avoid the side effects of nausea and vomiting; b) permit more accurate delivery of the appropriate dose. The distribution and elimination kinetics of misonidazole, as well as its penetration into the CNS, were studied in monkeys given an i.v. dose of the drug. Rhesus monkeys were fitted with an adaption of the subcutaneous Ommaya CSF reservoir which enabled both the blood and CSF kinetics to be studied. CSF, blood, and urine samples were collected and analyzed by a gas chromatographic method we developed which measures both misonidazole and its major metabolite (1-(2-nitroimidazol-1-yl)-2-,3 propandiol (RO5-9963) also known to selectively sensitize hypoxic cells to ionizing radiation. Single i.v. doses at 100 mg/kg and 200 mg/kg were administered respectively to 2 animals at each dose and CSF, blood, and urine samples collected for analysis. The results of this study to date have shown that a rapid peak plasma level is attained proportional to the i.v. dose with an elimination half-life of 4-6 hrs (resembling results obtained in baboons given the oral preparation). Similar levels were seen in the CSF after one hour. Disappearance from the CSF paralleled the disappearance of the drug from blood. Metabolite levels reached approximately 10-20% that of the parent drug in the serum.

Disposition of Imidazopyrazole (NSC-51143) in Rats

Disposition studies of ^{14}C-imidazopyrazole were continued. Plasma radioactivity levels following i.v. doses of 100, 250, or 500 mg/kg to rats were multiphasic with a distribution phase over the first 60 mins ($T_{1/2}$ = 15-45 min) followed by a phase of very gradual decline over the next 5 hours ($T_{1/2}$ = 90 to 240 min). Plasma levels of radioactivity were proportional to dose at all time intervals with the slope of the slower second phase increasing slightly with decreasing dose. Radioactivity was distributed to all tissues; however, concentrations greater than those in plasma were found only in stomach, kidney, and liver at the 100 mg/kg dose, in stomach and kidney at the 250 mg/kg dose, and in stomach at the 500 mg/kg dose. The majority of the radioactivity was excreted in the urine, with 21.9% and 67.8% of the administered dose excreted by 6 and 24 hours, respectively. Fecal elimination was significantly less and accounted for 11.1% of total dose at 120 hours compared to 74.3% in the urine. Four hours after dose administration (250 mg/kg, iv), 51% of the radioactivity excreted in the urine migrated on TLC plates as unchanged imidazopyrazole. The remainder of the radioactivity migrated in at least 9 different spots. A correlation between the sustained plasma levels and the long duration of action of imidazopyrazole was established. Drug concentrations equivalent to those found in plasma of rats between 15 mins and 8 hours after dosing inhibited the growth of L1210 cells *in vitro* by 80%. Concentrations of drug corresponding to plasma levels present *in vivo* between 8 to 20 hours after dosing resulted in greater than 50% inhibition.

Disposition Studies of Gallium Nitrate, NSC-15200, in Patients Undergoing Phase I Clinical Trials

As part of the phase I gallium nitrate study underway in the Medicine Branch of NCI, urine and blood samples are being analyzed from patients who received tracer amounts of ^{67}Ga with therapeutic doses of non-radiolabeled gallium. These studies thus far indicate that gallium has a two phase plasma decay curve, that the second phase decline is quite slow, that high dose gallium is predominantly excreted in the urine, and that the plasma gallium levels do not seem to increase linearly with increasing infused gallium doses. At the lowest gallium dose (20 mg/m^2) peak plasma levels were 7 μg/ml, while at

450 mg/m^2 the peak plasma levels were only 15-20 μg/ml, a doubling of plasma levels despite a 15-fold increase in dose. The data suggest that, at low doses, gallium may be bound and preferentially retained in the plasma compartment.

Publications

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4. Cysyk, R. L., Shoemaker, D. D., Ayers, O. C., and Adamson, R. H.: Oral absorption and selective tissue localization of 4'-(9-acridinyl-amino)-methanesulfon-m-anisidide. Pharmacology, 16: 206-213, 1978.

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7. Shoemaker, D. D., Legha, S. S., and Cysyk, R. L.: Selective localizaton of 4'-(9-acridinylamino)-methanesulfon-m-anisidide in B_{16} melanoma. Pharmacology 16: 221-225, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03509-15 LCHP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Carcinogenesis, Chemotherapy and Biological Markers in Non-Human Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The carcinogenic effects of 26 substances, including antineoplastic and immunosuppressive agents, contaminants of human foodstuffs, rodent carcinogens, pesticides, and artificial sweeteners are being evaluated in four species of non-human primates. Nineteen of these substances have not as yet demonstrated carcinogenic activity, although some have been on test for less than two years. Seven of the test compounds are carcinogenic in non-human primates, producing tumors in 13-100% of the treated animals. The effects of five of the seven compounds (DENA, DPNA, 1-nitrosopiperidine, aflatoxin B_1 and MAM-acetate) were manifested primarily as hepatocarcinogenicity. In addition, 1-methyl-1-nitrosourea induced squamous cell carcinomas of the oropharynx and esophagus, with the esophageal tumors in monkeys possessing many clinical and morphologic similarities to human esophageal carcinoma. Long-term treatment with procarbazine resulted in an increased incidence of malignancies, approximately one-half of which were acute myelogenous leukemia. Alpha-fetoprotein has been found to be a useful marker for diagnosing and for following the success of treatment of hepatocellular carcinoma in non-human primates. Studies on chemo-prevention of cancer in non-human primates using various nucleophiles are in progress.

Project Description:

Objectives:

To determine whether the non-human primate resembles the rodent in that it is more susceptible to chemical carcinogenesis as a neonate than as an adult.

To evaluate the carcinogenic potential and long-term adverse effects of clinically useful antineoplastic and immunosuppressive agents.

To develop model tumor systems in primates for evaluating the potential usefulness of new anticancer agents active against rodent tumors before these agents are administered to cancer patients.

To evaluate the possibility of preventing or reversing chemical carcinogenesis in non-human primates with nucleophiles or immunostimulation.

To obtain data on which to base a comparison of the response of non-human primates and rodents to materials known or suspected to be carcinogenic to man, and thereby to evaluate the relative merits of rodents and nonhuman primates in predicting for man.

To use normal and tumor-bearing non-human primates for studying the pharmacological, toxicological, and chemotherapeutic properties of various anticancer, antiviral, and antimicrobial agents.

To carry out comparative biochemical and immunological studies using normal animals, animals with preneoplastic lesions, and animals with malignant neoplasms.

To develop biological markers as an aid in detecting preneoplastic changes and for use in the early diagnosis of tumors.

Methods Employed:

Compounds Under Investigation:

Twenty-six substances are currently under investigation or have been investigated, including: 3-methylcholanthrene, dibenz(a,h)anthracene, 3,4,9,10-dibenzpyrene, N-2-fluorenylacetamide, N,N-2,7-fluorenylenebisacetamide, ethyl carbamate, N,N'-dimethyl-p-phenylazo-analine, N,N'-dimethyl-p-(m-tolylazo)-analine, methylnitrosourea, aflatoxin B_1, methylazoxymethanol acetate, cyclamate, saccharin, dichlorodiphenyltrichloroethane, N-methyl-N'-nitro-N-nitrosoguanidine, low density polyethylene plastic, cigarette tobacco smoke condensate, 1-nitrosopiperidine, N-nitrosodiethylamine, N-nitrosodipropylamine, arsenic, sterigmatocystin, Imuran, Adriamycin, procarbazine and L-phenylalanine mustard.

The compounds are administered subcutaneously, intravenously, intraperitoneally or orally. For oral administration to newborn monkeys, the compound is added to the Similac formula at the time of feeding; when the monkeys are six months old, carcinogens given orally are incorporated into a vitamin mixture which is given to monkeys as a vitamin sandwich on a half slice of bread. The dose level chosen is dependent on the chemical under evaluation. Antineoplastic and immunosuppressive agents are administered at doses likely to be encountered in a clinical situation; other substances, such as environmental contaminants, are given at levels 10-40 fold higher than the estimated human exposure level. The remainder of the chemicals tested are administered at maximally tolerated doses which, on the basis of weight gain, blood chemistry and hematology findings, and clinical observations, appear to be devoid of acute toxicity.

Animals

The present colony, consisting of approximately 600 animals, is comprised of four species: Macaca mulatta (rhesus), Macaca fasicularis (cynomolgus), Cercopithecus aethiops (African green) and Galago crassicaudatus (bushbabies). Eighty of these monkeys are adult breeders which supply the newborns for experimental studies. The majority of the animals are housed in an isolated facility which contains only animals committed to this study, and with the exception of the breeding colony, most animals are housed in individual cages. Newborns produced by the breeding colony are taken within 12 hours of birth to the nursery which is staffed on a 24 hour basis. The administration of test compounds is usually initiated within 24 hours of birth and continues until a tumor is diagnosed or until a predetermined exposure period has been completed. A minimum of 30 animals is usually allotted to each treatment group, since in a sample of this size it is possible to detect a tumor incidence of 10% within 95% confidence limits.

A variety of clinical, biochemical and hematological parameters are monitored weekly or monthly, not only to evaluate the general health status of each animal, but also for the early detection of tumors. Surgical procedures are performed under phenycylidine hydrochloride, Ketamine or sodium pentobarbital anesthesia. All animals which die or are sacrificed are carefully necropsied and the tissues subjected to histopathologic examination.

Major Findings:

Compounds Not Carcinogenic in Non-Human Primates

Since the inception of this study 16 years ago, 2 spontaneous tumors have been diagnosed in 211 non-treated breeders and vehicle-treated controls, yielding a tumor incidence of less than 1%. Of the 26 substances entered on test during this period, 19 (3-methylcholanthrene, dibenz(a,h)anthracene, 3,4,9,10-dibenzpyrene, N-2-fluorenylacetamide, N,N-2,7-fluorenylenebisacetamide, ethyl carbamate, N,N-dimethyl-p-phenylazoanaline, N,N'-dimethyl-p-(m-tolylazo)-analine, cyclamate, saccharin, dichlorodiphenyltrichloroethane; N-methyl-N'-nitro-N-nitrosoguanidine, low density polyethylene plastic, cigarette smoke condensate, arsenic, sterigmatocystin, Imuran, Adriamycin and L-phenylalanine mustard, have not as yet induced tumors. However, several of these compounds have been under evaluation for less than 2 years.

Compounds Carcinogenic in Non-Human Primates

Seven of the 26 substances evaluated are carcinogenic in non-human primates, producing a tumor incidence ranging from 13-100% of the treated animals. The carcinogens include N-nitrosodiethylamine, 1-nitrosopiperidine, N-nitrosodipropylamine, aflatoxin B_1, methylazoxymethanol acetate, procarbazine, and methylnitrosourea.

Nitrosamine Carcinogenesis

N-nitrosodiethylamine (DENA) has induced tumors in 32 out of 45 Old World monkeys receiving oral treatment with 40 mg/kg 5 times a week. These tumors, all of which were hepatocellular carcinomas, developed in rhesus, cynomolgus and African green monkeys which had received the initial dose of DENA either at birth, at 1-8 months postpartum, or as adults. The average latent period for tumor development was 38 months with a range of 14-148 months. The average total dose of DENA ingested by the monkeys developing tumor was 26.5 gm, and ranged from 6-58 grams. There is some indication that monkeys receiving the initial dose of DENA at birth required less total DENA for tumor development than those monkeys in which initial treatment was delayed until 1-8 months postpartum or until adulthood. However, the number of animals in each group is not sufficiently large to test the statistical significance of this apparent difference. DENA was also carcinogenic when given by the ip route. A total of 98 Old World monkeys given bimonthly doses (40 mg/kg) of DENA developed hepatocellular carcinomas. The average latent period was 17 months (range 6-32 months) and the average total dose of DENA necessary for tumor development was 1.7 grams (range 0.39-4.08 gm). The latent period and the total dose required for tumor development appeared to be independent of the age at which dosing was initiated as well as of the species and sex of the animals.

DENA is also carcinogenic in the more primative primate *Galago crassicaudatus*. Ten out of a total of 12 animals have developed tumors after bimonthly ip injections of DENA at doses of 10-30 mg/kg. In contrast to the DENA-induced primary hepatocellular carcinomas in Old World monkeys, all 10 of the bushbabies developed mucoepidermoid carcinomas of the nasal cavity. In 2 of these 10 animals, carcinoma of the liver was also present, and in both cases metastases to the lungs or to intestinal lymph nodes was noted. The average total dose of DENA given the bushbabies was 0.747 gm, and ranged from 0.295-1.485 gm. The latent period averaged 20 months (range 12-27 months).

There is no obvious reason for the marked difference noted between Old World monkeys and bushbabies with regard to the site of DENA-induced tumors. It is possible that it is related to differences between bushbabies and Old World monkeys in the metabolism and physiologic disposition of DENA, and this possibility is currently being investigated in our laboratory.

Two other nitrosamines have induced primary hepatocellular carcinomas in monkeys. N-nitrosodipropylamine (DPNA) induced liver tumors in all 6 of the rhesus and cynomolgus monkeys given bimonthly ip doses of 40 mg/kg. The average total dose of DPNA was 7.0 gm; the average latent period for tumor development was 28.5 months. 1-nitrosopiperidine (PIP) is also an hepatocarcinogen in macaques. Hepatic cell carcinomas developed in 11 of 12 monkeys receiving this compound by the oral route, and in 4 out of 11 monkeys treated by the ip route. The average cumulative dose necessary for tumor induction by PIP given orally (1706.1 gm) was higher than for oral DENA (26.5 gm); similarly, the average cumulative dose of PIP given by the ip route (60.95 gm) exceeded that required for tumor induction by ip DENA (1.71 gm) or ip DPNA (7.0 gm).

Carcinogenic Activity of Aflatoxin B_1 and Methylazoxymethanol Acetate

Aflatoxin B_1 (AFB_1) and methylazoxymethanol acetate (MAM-acetate), two substances known to be contaminants of human foodstuffs, are carcinogenic in non-human primates. Six of 45 monkeys (13%) treated with aflatoxin B_1 for longer than 2 years have developed tumors. Three of the tumors were primary liver carcinomas, and single cases of hemangioendothelial sarcoma of the liver, olfactory neuroepithelioma, and osteosarcoma of the tibia were diagnosed. The latent period for tumor induction ranged from 48 months to 118 months, averaging 76 months; the average total dose given to monkeys developing tumors was 344.5 mg (range 99-842 mg).

MAM-acetate, the aglycone of the active carcinogenic compound in the cycad nut, cycasin, also induces liver carcinomas in monkeys. A total of 26 rhesus, cynomolgus and African green monkeys were given MAM-acetate by weekly ip injections (10 mg/kg) or were given compound orally (3 mg/kg, 5 days every week). To date 7 animals (27%) have developed primary liver cancer; in one of these monkeys a liver hemangiosarcoma, bilateral renal carcinomas and a squamous cell carcinoma of the esophagus was also found. When administered by the ip route the average latent period for tumor development was 81 months (ranging from 50-100 months), and the average total dose received was 6.1 grams (ranging from 3.58-9.66 gm).

Carcinogenic Activity of Methylnitrosourea

Squamous cell carcinoma of the mouth, pharynx, and esophagus developed in 6 of 43 monkeys (14%) receiving oral doses (10-20 mg/kg) of 1-methyl-1-nitrosourea (MNU). Moreover, upper digestive tract lesions such as atrophy or dyskeratosis of the esophageal mucosa and esophagitis have been a consistent finding among the 18 monkeys necropsied to date. All monkeys except one that have received total doses of MNU exceeding 50 grams have developed carcinomas, whereas no malignant tumors have developed in monkeys receiving a cumulative dose less than 50 grams. The average latent period for tumor development was 78 months and ranged between 63-124 months. The order of appearance of the esophageal lesions in our monkeys, as well as the clinical manifestation of the tumors resembled those seen in humans, and included difficulty in swallowing, frequent vomiting and subsequent weight loss, and sialorrhea. The common complications of esophageal carcinoma in humans (e.g., regurgitation, aspiration, sepsis and hemorrhage) were also noted in our monkeys. In addition, histological examination revealed a morphology similar to that seen in human esophageal carcinomas, despite the highly variable nature of such tumors in both human and monkeys. Thus, MNU-induced esophageal carcinoma in non-human primates may prove to be a valuable model for the study of the human tumor.

Carcinogenic Activity of Procarbazine

Fifty monkeys have survived for longer than 6 months after receiving treatment with procarbazine by sc, ip and/or oral routes at doses of 5-50 mg/kg. Thirty-seven monkeys have been necropsied to date, of which 11 (22%) have had malignant neoplasms. Six monkeys were diagnosed with acute leukemia, all but one of the myelogenous type; the other acute leukemia was undifferentiated. In addition to the leukemias, two cases of hemangiosarcoma, two cases of osteogenic sarcoma, and one case of lymphocytic lymphoma, was found. The neoplasms were diagnosed after procarbazine treatment for an average of 75 months (ranging from 16 to 109 months); the total dose of procarbazine received by the monkeys developing tumors ranged from 2.69-101.6 gm, and averaged 36.0 gm. Other adverse effects of long-term procarbazine treatment included vomiting, myelosuppression and testicular atrophy with complete aplasia of the germinal epithelium.

DENA Dose-Response Studies and Attempts at Chemoprevention

DENA is highly predictable as a hepatocarcinogen in Old World monkeys, and accordingly we have accumulated a relatively large amount of information on its carcinogenic effects in these animals. We are therefore currently using this chemical as a model carcinogen to investigate two questions of importance to chemical carcinogenesis.

The first question pertains to whether there is a specific total dose of carcinogen which, within the lifespan of the test animal, will induce a tumor. In order to evaluate this question, groups of monkeys are being given bimonthly ip injections of DENA at doses of 1, 5, 10, 20 and 40 mg/kg, and are observed for the appearance of tumor. In the 4 groups of monkeys (40, 20, 10 and 5 mg/kg doses) in which tumors have developed, we have found that the latent period increases as the mg/kg dose decreases. Thus with the 40 mg/kg dose the latent period is 17 months, whereas at 5 mg/kg it is 55 months. The study is as yet incomplete and in some groups the proportion of tumor-bearing animals is small, so that it is not yet possible to report a precise value for the carcinogenic dose of DENA. However, it appears that this value will lie between 1.2 and 2.2 gm.

The second question pertains to whether chemically-induced cancer can be prevented or reversed by other chemicals, either administered simultaneously with the carcinogen, or before carcinogen exposure. It is now recognized that the reactive forms of most chemical carcinogens are strong electrophiles, and are capable of attacking tissue nucleophiles. Thus, one of the possible approaches to the prevention or reversal of chemical carcinogenesis is to supply an excess of non-critical nucleophiles, thereby "scavenging" the reactive electrophilic species of carcinogens once formed. We are using this approach in 2 groups of monkeys receiving bimonthly ip doses of DENA at 10 mg/kg or 5 mg/kg. In both groups, the DENA dose is given 10 minutes after an ip injection of a "protective cocktail". For the monkeys receiving DENA at 10 mg/kg, the "protective cocktail" consists of l-cysteine (20 mg/kg), cysteamine (20 mg/kg) and reduced glutathione (150 mg/kg). The "protective cocktail" given the monkeys receiving DENA at 5 mg/kg is composed of N-acetylcysteine (50 mg/kg). These substances were chosen as nucleophilic chemoprotectants for two reasons. First, they do not reduce the antitumor effects of various clinically useful alkylating agents and therefore could be used to protect against the carcinogenicity of these drugs without interfering with their therapeutic effects. Secondly, both N-acetyl-cysteine and cysteamine have been shown to prevent the covalent binding of acetaminophen to tissue, and for this reason have been used in humans to reverse the hepatotoxic effects of acute acetaminophen poisoning. By analogy, it is hoped that these sulfhydryl reagents will prevent the covalent binding of the activated form of DENA to critical macromolecules of liver tissue, an event which is thought to be a prerequisite for hepatocarcinogenesis by a variety of chemicals including DENA. To date, none of the monkeys have received the minimum cumulative dose of DENA (1.2 gm) required for tumor development. The monkeys on this study longest are those receiving DENA at 10 mg/kg. In this group, the average cumulative DENA dose to date is 0.759 gm, and the highest total dose given a monkey is 0.950 gm.

Alpha-Fetoprotein (AFP) Studies

Using radio-immunoassay, the serum AFP levels in monkeys with biopsy-proven liver tumor showed a 100,000-fold variation, which is analogous to the range seen in the serum of humans with hepatocellular carcinoma. Monkeys with N-nitrosodiethylamine-induced neoplasms generally had significantly higher serum AFP levels than those with tumors induced by l-nitrosopiperidine, cycasin or aflatoxin B_1. Serial determinations in monkeys with liver tumor demonstrated a tendency for AFP levels in each animal to plateau after reaching a level characteristic for that tumor.

The half-life of native serum AFP was measured under three different conditions: serum from hepatoma-bearing monkeys injected i.v. into normal adult monkeys, the post-partum decline in serum AFP levels in neonatal monkeys, and the decline in AFP levels in hepatoma-bearing primates following surgical removal of the tumor. Despite many differences among these situations, the results tended to corroborate a T 1/2 for AFP of 2.8-3.6 days.

Studies on the *in vitro* synthesis of AFP were completed. Five monkeys were treated ip with N-nitrosodiethylamine (DENA), and one was treated with 1-nitrosopiperidine (PIP), starting within 2 months of birth, until hepatocellular carcinoma (HCC) developed. All animals except the PIP-treated monkey had markedly elevated serum AFP values. Fresh, minced, biopsy-derived tumor was cultured with L-[^{14}C]lysine. Synthesis of AFP was determined by radioimmunoassay and by specifically precipitable [^{14}C]AFP. Good agreement between these two parameters was obtained for the 4 DENA-induced tumors synthesizing AFP in culture. Tumor from 1 DENA-treated monkey did not synthesize AFP. In addition, neither normal liver nor tumor from the PIP treated monkey synthesized AFP. Rates of synthesis were 0.37-5.50 ng AFP/mg tumor/day, or 0.0012-0.0183 pg AFP/cell/day (if one assumes 3.0×10^5 cells/mg tissue) over 48 or 72 hours. Different nodules from the same animal had similar rates of synthesis. For tumors that synthesized AFP in culture, a positive correlation was generally found between rate of synthesis and serum AFP level. The rate of *in vitro* AFP synthesis observed was lower than that of immunoglobulin synthesis in human myeloma or of AFP synthesis in a rat HCC, but it was close to the estimated rate of AFP synthesis in a monkey HCC line in long-term culture.

Therapy

Surgery is the only modality which has consistently effected a 50% reduction in the mass of primary liver tumors *and* serum AFP levels; however, several chemotherapeutic agents have reduced either the tumor mass or the AFP level by more than 50% in some animals. For example, in one monkey, three courses of adriamycin at 1 mg/kg over a 6 month period reduced the tumor mass and decreased the serum AFP from 252,000 ng/ml to 10,000 ng/ml. In addition, BCG in two monkeys has thus far either delayed or prevented the induction of tumor by nitrosamines. Other chemotherapeutic agents (poly I·poly C, methotrexate, 6-MP, 5-FU gallium nitrate) have been tested, but responses were obtained only with methotrexate and gallium.

Two new chemotherapeutic agents, PALA and m-AMSA are under evaluation in non-human primates for activity against chemically-induced primary liver tumors. In addition, the systemic toxicity and immune-stimulatory properties of a vaccine made from BCG cell walls is being evaluated as well as its therapeutic effect following intra-tumor injections.

Publications:

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2. Adamson, R. H., Krolikowski, F. J., Correa, P., Sieber, S. M. and Dalgard, D. W.: Carcinogenicity of 1-methyl-1-nitrosourea in nonhuman primates. J. Natl. Cancer Inst. 59: 415-422, 1977.

3. Sieber, S. M. and Adamson, R. H.: Discussion: Some comments on the potential carcinogenicity of the clinically useful antitumor agents. Cancer 40: 1950-1951, 1977.

4. Thorgeirsson, S. S., Sakai, S. and Adamson, R. H.: Induction of monooxygenases in rhesus monkeys by 3-methylcholanthrene: metabolism and mutagenic activation of N-2-acetylaminofluorene and benzo(a)pyrene. J. Natl. Cancer Inst. 60: 365-369, 1978.

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7. Sieber, S. M., Correa, P., Dalgard, D. W. and Adamson, R. H.: Carcinogenic and other adverse effects of procarbazine in non-human primates. Cancer Res., 1978, in press.

8. Adamson, R. H. and Sieber, S. M.: Chemical carcinogenesis in non-human primates. In Proceedings of the Workshop on Non-Human Primates in Cancer Research, 1978, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06108-09 LCHP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on Mechanism of Action and Mechanisms of Resistance of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. L. Cysyk	Pharmacologist	LCHP NCI
OTHERS:	P. E. Gormley	Medical Officer	LCHP NCI
	D. D. Shoemaker	Staff Fellow	LCHP NCI

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Drug Metabolism Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	1.5	1.5

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A Lewis Lung carcinoma line resistant to PALA (NSC-224131) was developed as a tool to elucidate the biochemical basis for the unique spectrum of antitumor activity exhibited by this agent. In a continuing study, additional experiments were performed to determine the mechanism of cytotoxicity of AMSA (NSC-141549) and to determine the basis for its in vivo selective toxicity. Rat liver glutathione-S-transferases were purified to homogeneity and the properties of these enzymes from developing mouse tissues studied as a basis for understanding the selective toxicity exhibited by certain antitumor agents. A limited study was performed on the interaction of two new anthracenedione derivatives (NSC-279836 and NSC-287513) with DNA and the resultant effect on the template activity of DNA as determined by inhibition of DNA-dependent RNA polymerase. Several new 5'-methylphosphonate analogues of anti-tumor pyrimidine mononucleotides are being synthesized based on the results from a biologic study of ara-C-5'-methylphosphonate.

Project Description:

Objectives:

A knowledge of the mechanism of action of anticancer agents and why cells become resistant to them will aid in the design of new chemotherapeutic drugs and will aid in the treatment of neoplastic diseases. Specifically:

To determine the mechanism of action of various anticancer agents.

To determine the mechanism of resistance to various anticancer agents.

To rationally design new anticancer agents.

Methods Employed:

Usual biologic, pharmacologic, and biochemical techniques. Among these are tumor transplantation, harvesting of cells, tissue culture techniques, separation and analyses of whole cell DNA, RNA, and protein synthesis. Various enzymes are isolated, purified and the effect of various antitumor agents as inhibitors of these enzymes are studied as well as effects on DNA, RNA and various proteins. Molecular models are also used in drug design.

Major Findings:

Studies on N-(Phosphonacetyl)-L-Aspartate (NSC-224131, PALA)

PALA, an agent presently undergoing clinical evaluation, was initially synthesized as an analogue of the transition state for the aspartate transcarbamylase (ATCase) reaction. This agent has an unusual spectrum of activity in that it is highly active against certain solid tumors (including Lewis Lung carcinoma, B16 melanoma, glioma 26) but has virtually no activity against various fast growing tumors (including leukemias L1210, P388, L5178Y). Furthermore, it has been reported by others that the difference in sensitivity of these tumors is due to a 2-fold increase in ATCase in the insensitive tumor lines. Since the ultimate pool sizes of pyrimidines is determined by both the *de novo* and salvage pathways, we decided to determine if differences in the activity of the salvage pathway could account for differences in tumor sensitivity to PALA. For this purpose, uridine kinase was selected as an indicator of the integrity of the salvage pathway and also because uridine is known to reverse the anti-tumor effects of PALA. Uridine kinase activity was determined as a function of tumor growth in 8 tumors that had sensitivities to PALA ranging from totally insensitive to completely responsive. There was no consistent relationship between uridine kinase activity and PALA resistance, although in the totally resistant leukemic lines (L1210 and P388) there was a higher level of enzyme activity. In the slow growing solid tumors, however, uridine kinase activity appeared to be more directly related to proliferation rate and stage of growth than to PALA insensitivity. Uridine kinase activity must contribute to some extent to PALA resistance, however, since the normally unresponsive L1210 became partially responsive to

PALA after pretreatment of this tumor with Ara-C for several generations to decrease uridine kinase levels by 80-90%. However, experiments in which mice bearing L1210 were treated sequentially with Ara-C for 4 days (to select cells with decreased kinase activity and therefore more dependent on the de novo pathway) followed by PALA for 4 days (to exploit the dependence on the de novo pathway) indicated no enhancement of effect over that of Ara-C alone. Such drug combination experiments will be continued to establish rational drug combinations with PALA for possible clinical application. Efforts were undertaken to develop a PALA-resistant subline of Lewis Lung carcinoma (the tumor line most sensitive to PALA) in order to study the biochemical mechanism of resistance. This has been accomplished, and the resultant line is totally resistant (in terms of tumor growth and median survival time) to a dose of 200 mg/kg on 4 consecutive days. In contrast, with the sensitive Lewis Lung carcinoma line 8/8 cures are obtained with a dose of 100 mg/kg, and 5/8 cures are obtained with a 50 mg/kg dose. A comparison of normal and resistant lines revealed no differences in uridine kinase activity, indicating that resistance is not due to an enhanced salvage pathway. Moreover, a 10-30% increase in ATCase activity noted in the resistant subline was not sufficient to account for the total resistance of this line to extremely high doses of PALA. C^{14}-PALA concentrations in the resistant line were 10-40% lower than in the normal line after a single dose of 100 mg/kg; again, this is not a sufficient difference to fully account for the resistance since a dose of 200 mg/kg is ineffective against the resistant line. Elucidation of the mechanism of resistance to PALA might provide useful information on the unusual spectrum of its anti-tumor activity as well as on its mechanism of action. Equally important are the clinical application of such findings as they might predict which patients should benefit from PALA treatment and which drugs might be combined with PALA to exploit the biochemical lesion that results in PALA resistance.

Studies on the Mechanism of Action of 4'-(9-acridinylamino)-methanesulphon-m-anisidide (AMSA, NSC-141549).

Studies on the interaction of AMSA and DNA were continued (see previous annual report). In a comparative study, proflavine and 9-aminoacridine were found to bind to DNA as well as or better than AMSA as determined by a fluorescence technique and by alteration of DNA thermal stability. Also, studies with DNA and RNA polymerases revealed that proflavine and 9-aminoacridine were better inhibitors of these enzymes than was AMSA. The results emphasize that many acridine compounds besides AMSA have considerable biological activity and, before a mechanism of action can be assigned to AMSA, the mechanism must be shown to be, in some way, unique to AMSA and not shared by the general class of aminoacridines. O-AMSA is an analogue of AMSA in which the methoxy group in the meta position of the anisidide side chain of AMSA is in the ortho position. In contrast to AMSA, O-AMSA was found to be inactive as an anti-tumor agent and approximately 10% as cytotoxic as AMSA against L1210 cells in vitro. O-AMSA is as susceptible to thiolysis as is AMSA, binds to DNA, but is 50 to 75% less inhibitory to DNA-dependent RNA polymerase. A more detailed comparative investigation of the biological effects of O-AMSA and AMSA should yield valuable information as to the mechanism of action of AMSA. Present studies are examining the properties of DNA isolated from tumors and certain normal tissues following AMSA treatment, with special emphasis on the effects of AMSA metabolites on the integrity and biologic properties of DNA.

Studies on the Selective Toxicity of AMSA (NSC-141549)

In the annual report of last year we postulated that the selective toxicity of AMSA might be related to differences in detoxification by tumors and certain normal tissues. That glutathione functions in the detoxification of AMSA is evidenced by the following: identification of the major biliary metabolite as the glutathione-acridine thioether; reduction in liver glutathione levels following AMSA treatment; and an enhancement of AMSA toxicity in mice pretreated with a dose of diethylmaleate that produces a 80% reduction in liver glutathione. AMSA was found to interact with glutathione-S-transferase as determined by inhibition of this enzyme at AMSA concentrations of $1x10^{-5}$M. Glutathione-S-transferases from rat liver were purified to homogeneity and were found not to irreversibly bind AMSA as determined by Sephadex gel filtration. The substrate capacity of AMSA for these enzymes is presently under investigation. The glutathione-S-transferases are a family of soluble proteins involved with the detoxification of exogenous compounds, e.g., the reactive metabolites of carcinogenic azo dyes and polycyclic hydrocarbons. These enzymes appear to play an important role in the metabolism and disposition of AMSA and possibly for other antitumor agents as well. Studies are underway to characterize this class of enzymes in the CDF_1 mouse, a laboratory animal commonly used in testing the cytotoxicity of antitumor agents. The activity of glutathione-S-transferases in the newborn CDF_1 mouse were found to be approximately 10% that of the adult and to gradually increase with postnatal development, reaching adult levels in 8 weeks. In adult mice the activity was shown to be inducible by phenobarbital and to vary with substrate, as enhanced activities of 60% and 100% were demonstrated for 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene, respectively, after one week of phenobarbital treatment. Purification studies are in progress to determine if the mouse glutathione-S-transferase activity resides in a family of distinct, differently charged species or in one single enzyme. In other studies in tumors it was found that glutathione-S-transferase activity might be directly related to the extent of tumor differentiation since this enzyme constitutes 5-10% of normal monkey liver protein but is barely detectable in monkey hepatocellular carcinoma. Samples of human hepatocellular carcinoma and liver metastases were also found to be lacking these enzymes. Attempts to exploit this difference for the treatment of hepatocellular carcinoma and liver metastases are presently being made with AMSA and other anti-tumor agents that interact with, and are detoxified by, glutathione-S-transferase. A model system is being evaluated in which tumor cells are surgically implanted into the livers of rats and the animals then treated with agents that were previously selected on the basis of their interaction with glutathione-S-transferase.

Studies on Two New Anthracenedione Derivatives (NSC-279836 and NSC-287513)

Two new anthracenedione derivatives (NSC-279836 and NSC-287513) are highly active against a variety of animal tumors and are being considered by the NCI for high priority development toward clinical trials. These compounds were tested for DNA interaction and possible effects on nucleic acid polymerizing enzymes as a prelude to a more detailed study into their mechanism(s) of selective toxicity. Each compound was found to bind tightly to calf thymus DNA (as anticipated from their multi-ring structure), as determined by alteration in the UV and visible absorption spectra. Also, the template function of the DNA-drug complexes was found to be altered as determined by inhibition of DNA-dependent RNA polymerase activity. Enzyme activity was inhibited 50% at 20 ug/ml and 100% at drug concentrations greater than 100 ug/ml. This compares with concentrations of AMSA of 200-400 ug/ml necessary for 50% inhibition in the same system.

Synthesis and Biologic Activity of 5'-Methylphosphonate Analogues of Nucleoside Monophosphates

The 5'-methylphosphonate analogue of AraCMP was synthesized in an attempt to circumvent resistance that develops to Ara-C as a result of deletion of CdR kinase (see previous annual report). We found that although Ara-C-5'-methylphosphonate (Ara-CMeP) inhibits dCMP kinase as effectively as Ara-CMP, Ara-CMeP is not phosphorylated to the nucleoside diphosphate form. This indicated that because of its structural similarity in the pyrimidine portion of the molecule, Ara-CMeP can bind as well as Ara-CMP to dCMP kinase; however, methylation of the phosphate moiety alters the nucleophilicity of this portion of the molecule such that phosphorylation does not occur. Based on this information we are presently synthesizing 5'-methylphosphonate analogues of other pyrimidine nucleoside anti-tumor agents that are active as the monophosphate form and thus would be resistant to successive phosphorylation and to phosphatase attack.

Publications:

1. Sinha, B. K., Philen, R. P., Sato, R., and Cysyk, R. L.: Synthesis and antitumor properties of bis-quinaldine derivatives. J. Med. Chem. 20: 1528-1531, 1977.

2. Gormley, P. E., Benvenuto, J., and Cysyk, R. L.: Synthesis and biologic studies of arabinosylcytosine 5'-methylphosphonate. Biochem. Pharmacol. 26: 1291-1294, 1977.

3. Cysyk, R. L., Shoemaker, D., and Adamson, R. H.: The pharmacologic disposition of 4'-(9-acridinylamino)methanesulfon-m-anisidide in mice and rats. Drug Metab. Disp. 5: 579-590, 1977.

4. Cysyk, R. L., Shoemaker, D. D., Ayers, O. C. and Adamson, R. H.: Oral absorption and selective tissue localization of 4'-(9-acridinylamino)-methanesulfon-m-anisidide. Pharmacology 16: 206-213, 1978.

5. Shoemaker, D. D., Legha, S. S., and Cysyk, R. L.: Selective localizaton of 4'-(9-acridinylamino)-methanesulfon-m-anisidide in B_{16} melanoma. Pharmacology 16: 221-225, 1978.

6. Cysyk, R.L., Gormley, P. E., D'Anna, M. E. and Adamson, R. H.: The disposition of 3-deazauridine in mice. Drug Metab. Dispos. 6: 125-132, 1978.

7. Gormley, P. E., Sethi, V. S., and Cysyk, R. L.: Interaction of 4'-(9-acridinylamino)-methanesulfon-m-anisidide (AMSA) with DNA and inhibition of oncornavirus reverse transcriptase and cellular nucleic acid polymerases. Cancer Research 38: 1300-1306, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06134-03 LCHP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Role of the Lymphatic System in the Absorption and Distribution of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Sieber-Fabro	Pharmacologist	LCHP	NCI
OTHER:	R. H. Adamson	Chief, Lab. of Chem. Pharm.	LCHP	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Pharmacology and Experimental Therapeutics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of the lymphatic system in the absorption and distribution of a variety of radiolabelled antitumor agents (CCNU, methyl-CCNU, guanazole, dibromomannitol, diethylstilbestrol, prednisolone, dexamethasone, 6-mercaptopurine, cytosine arabinoside, vincristine, cyclophosphamide, adriamycin, daunomycin, hexamethylmelamine, procarbazine, actinomycin D and o,p-DDD) in rats is under investigation. The relationship between extent of lymphatic absorption and the molecular weight and lipid solubility of the agents, their route of administration, and the vehicle or formulation in which they are given, is also being studied. Efforts are underway to develop a reproducible model system in rats for tumor metastasis via lymphatic channels, thus making it possible to evaluate the therapeutic advantage of treating such tumors with antitumor agents selectively taken up by lymphatics. The physiologic disposition of the plant-derived antitumor agent, maytansine is under investigation in rats and in humans.

Project Description:

Objectives:

This project was initiated in order to provide information on the role of the lymphatic system in the absorption and distribution of a wide variety of clinically useful antitumor agents following administration of these drugs by intraduodenal, intravenous or intraperitoneal routes. The goal of such studies is to determine whether there are specific physico-chemical characteristics which a compound must possess to ensure its preferential absorption and/or distribution by lymphatic vessels. These studies are designed to determine whether the lymphatic uptake of a compound can be influenced by the route of its administration and by the vehicle or formulation in which it is given. Particular emphasis is being placed on the relationship of the molecular size of a substance to the extent of its lymphatic absorption from the peritoneal cavity. Among the substances under investigation in this phase of the study are antitumor agents encapsulated in phospholipid vesicles of varying size and chemical composition. The relative rates of removal from the peritoneal cavity by lymphatic versus blood routes is being examined for liposome-encapsulated antitumor agents and for the respective un-encapsulated drugs.

Efforts are underway to develop a reproducible model system in rats for tumor metastasis via lymphatic channels, thus making it possible to evaluate the therapeutic advantage of treating such tumors with antitumor agents selectively taken up by lymphatics.

The physiologic disposition in rats and in humans of the plant-derived antitumor agent, maytansine, is under investigation.

Major Findings:

The intestinal absorption of most of the antitumor agents tested to date takes place primarily via the portal route rather than by the intestinal lymphatic system. Following the intraduodenal administration of radioactive CCNU, methyl-CCNU, guanazole, dibromomannitol, diethylstilbestrol, prednisolone, dexamethasone, 6-mercaptopurine, cytosine arabinoside, and cyclophosphamide, 5% or less of the administered radioactivity was recovered in 24-hour thoracic duct lymph. In lymph from rats treated with ^{14}C-6-mercaptopurine, approximately 20% of the radioactivity was associated with the parent compound; similarly, approximately 53% of the ^{14}C-activity in lymph from rats treated with radioactive cytoxan was identified as unchanged cytoxan. The only antitumor agent tested so far which appears to be selectively absorbed by the intestinal lymphatics is o,p-DDD. Following the administration of this compound in aqueous solution, 12.2% of the administered radioactivity was recovered in thoracic duct lymph, 73-81% of which was identified as unchanged o,p-DDD. When ^{14}C-o,p-DDD was administered as a suspension in corn oil, recovery of ^{14}C-activity in thoracic duct lymph was increased to 26.1% of the administered dose. An increase in lymphatic absorption of other compounds (prednisolone, dexamethasone, and 6-mercaptopurine) was also noted when they were administered as emulsions in corn oil rather than in aqueous solution, but this increase was not statistically significant. Other small molecular weight compounds of relatively high lipid solubility (CCNU, methyl-CCNU, diethylstilbestrol) were absorbed to approximately the same extent as were the relatively polar compounds (guanazole, cytosine arabinoside), regardless of whether they were given in aqueous solution or oil suspension. Compounds of relatively high molecular weight (vincristine, actinomycin D) were not more extensively taken up by lymphatic vessels than the lower molecular weight compounds. Approximately 0.4% of the administered dose of radioactivity was recovered in thoracic duct lymph of rats treated by either the intravenous or intramuscular routes with ^{3}H-vincristine. In contrast, 1.6% of the administered radioactivity was recovered in lymph from rats given ^{3}H-actinomycin D by the intramuscular route, whereas this value was increased 4-fold when the drug was given intravenously. Approximately equal amounts of cyclophosphamide equivalents (1.4-1.8% of the administered dose) were recovered in 24-hour thoracic duct lymph following the administration of ^{14}C-cyclophosphamide by intraduodenal or intravenous routes, suggesting that, at least in the case of cyclophosphamide, the lymph radioactivity derives from the distribution of cyclophosphamide equivalents in the body water, of which lymph is a part.

Studies in which the lymphatic absorption of intraduodenally or intravenously administered compounds has been measured have been carried out in rats in which the thoracic duct was cannulated by an abdominal approach. For studies of peritoneal absorption, however, it was necessary to develop a new surgical technique for cannulating the thoracic duct which left the peritoneal cavity intact. A method for such a surgical preparation has now been developed in which the thoracic duct is cannulated via a retrosternal approach, and studies of the role of the lymphatics in absorption of substances from the peritoneal cavity are underway. When ^{14}C-DDT or ^{14}C-DDA were given intraduodenally, 16% and 7%, respectively, of the administered radioactivity was recovered in thoracic duct lymph. By comparison, when ^{14}C-DDT was given by the intraperitoneal route, 2% of the administered ^{14}C-activity was recovered in thoracic duct lymph, and this value for ^{14}C-DDA was 7%.

Efforts to prepare phospholipid vesicles (liposomes) in which small molecular weight substances such as antitumor agents are encapsulated, are in progress. In preliminary studies, small unilamellar liposomes composed of phosphatidyl choline and ^{3}H-cholesterol (molar ratio 3:1) have been prepared which contain a trace amount of ^{14}C-glucose. Between 0.5-3% of the added glucose was encapsulated, and efforts are currently being aimed at improving this entrappment rate. Studies on the *in vivo* stability of such liposomes and their physiological disposition, including lymphatic absorption following intraperitoneal administration, are in progress. Such studies are a necessary prerequisite for further work on the lymphatic absorption of intraperitoneally administered antitumor encapsulated in liposomes of varying size.

Studies on the physiologic disposition of maytansine have been initiated. Rats given an iv injection of ^{3}H-maytansine excreted 61% and 6% of the administered radioactivity in 24-hour bile and urine, respectively. Less than 1% of the ^{3}H-activity was recovered in 24-hour thoracic duct lymph. Tritium activity disappeared from plasma with half-times of 54 minutes and > 24 hours for the alpha and beta phases, respectively. Preliminary results, using thin-layer chromatography, suggest that approximately 18% of the radioactivity in bile is attributable to unchanged maytansine. ^{3}H-maytansine was given as a 20-minute infusion to a patient with pancreatic carcinoma in whom a biliary fistula had been established. Radioactivity in samples of blood, bile and urine was monitored at intervals for 20 hours after the end of the infusion. Bile contained 28% of the administered tritium activity, and 45% of the radioactivity was recovered in urine. Tritium activity disappeared from plasma with half-times of 200 minutes and 16 hours for the alpha and beta phases, respectively.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06136-02 LCHP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Chemically Induced Cell Differentiation in Neoplasia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. S. Thorgeirsson	Head, Biochemical Pharma. Sect.	LCHP	NCI
OTHER:	P. J. Wirth	Staff Fellow	LCHP	NCI
	H. A. J. Schut	Cancer Expert	LCHP	NCI

COOPERATING UNITS (if any)

None

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NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was initiated to understand the mechanism of re-differentiation of neoplastic cells by various chemical agents. The research is directed at present to determine: (1) the effect of dimethylsulfoxide (DMSO) and its analogs on the expression of the enzymes in the γ-glutamyl cycle in hepatoma cell lines in culture, transplantable hepatomas as well as the Friend erythroleukemia cell line; (2) the effect of polar compounds such as DMSO, bisacetamides and antitumor agents on fetal expressions (such as α-feto-protein) in hepatomas and CEA in colon tumors; (3) the reversibility, when exposed to the above mentioned agents, of choriocarcinoma in tissue culture using HCG as marker.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The objective of this project is to examine the feasibility of using chemically induced cell differentiation as a therapeutic approach to neoplastic diseases either alone or in combination with established chemotherapeutic agents. This approach is aimed at finding chemicals with the capacity to "redifferentiate" or normalize transformed malignant cells. Information already available indicates that this approach is feasible. For example, the addition of DMSO to cultures of murine-virus induced erythroleukemia cells results in erythroid differentiation characterized by the appearance of globin mRNA, synthesis of hemoglobin, cessation of cell division, appearance of erythrocyte membrane antigens, and the morphological changes characteristic of erythroid differentiation in normal mouse hemopoietic tissue.

Methods Employed:

Methods used in these studies include: tissue culture technique, differential centrifugation, radioisotope measurements using tritium and carbon-14; phase microscopy; enzyme assays involving radiometric spectrophotometric and spectrophotofluorometric determinations; polyacrylamide gel electrophoresis; and chemical synthesis.

Major Findings:

1. Glutathione synthesis and degradation in fetal and adult rat liver and Novikoff Hepatoma. The *in vitro* activities of the three main enzymes involved in the synthesis and degradation of glutathione (γ-glutamylcysteine synthetase, glutathione synthetase and γ-glutamyltranspeptidase) were studied in the normal adult rat liver, in the fetal rat liver, and in the transplantable, rapidly growing, undifferentiated Novikoff hepatoma. The total glutathione (GSH) content in the Novikoff hepatoma and fetal rat liver was similar and both tissues had significantly ($p < 0.05$) lower GSH content than adult rat liver. GSH was rapidly depleted *in vivo* following the i.p. administration of diethyl maleate to male Sprague-Dawley rats with and without transplanted Novikoff hepatomas. Liver GSH levels in adult control rats were rapidly decreased to 15% of control levels 30 min after diethyl maleate administration, remained maximally depleted for four hr after which they began to rise rapidly, returning to normal values at 6 hr and to 200% of normal at 24 hr. GSH levels in the transplanted tumors, however, were maximally depleted (23% of normal) after 4 hr and showed a much slower rate of synthesis reaching normal levels only after 24 hr. Similarly, GSH levels in the fetal rat liver were depleted to 25% of control levels after 3 hr but rose to control values after 9 hr; unlike the adult rat liver and like the Novikoff hepatoma, the overshoot phenonenom for the resynthesis of GHS was not observed.

The *in vitro* activity of γ-glutamyltranspeptidase in the Novikoff hepatoma and in the fetal liver were markedly increased (50 and 16-fold respectively) over the transpeptidase activity in the adult rat liver. In vitro γ-glutamylcysteine synthetase activity in the adult control liver was 3-4 times greater than the synthetase activity in either the fetal liver or in the Novikoff hepatoma. The synthesis of GHS however, was 14 times greater in the adult liver than in the Novikoff hepatoma, and 3 times greater than in the fetal liver. GSH (5mM) inhibited the activity of γ-glutamylcysteine synthetase to the same degree in the adult liver, fetal liver, and Novikoff hepatoma, suggesting that the synthetase has similar enzymatic characteristics in all the tissues.

2. Cell culture. We have now characterized three hepatoma cell lines, namely, Novikoff, Morris (5123TC), and Reuber (H-4-II-E) with respect to the enzymes in the γ-glutamyl cycle. We have in addition to the hepatoma cell lines, established the Friend erythroleukemia cells and choriocarcinoma cells in our cell culture.

Significance to Biomedical Research and the Program of the Institute:

Our studies as well as those of others indicate that polar compounds such as DMSO can induce the neoplastic cell to "redifferentiate" and perhaps loose its malignant characteristics. Furthermore, the observations that glutathione synthesis is much slower in the neoplastic cell, may provide a way to increase the selective toxicity of some therapeutically used alkylating agents. For example, glutathione (GSH) can be depleted in both normal and neoplastic tissues by a variety of agents. Since the synthesis of GSH is much slower in the neoplastic tissue, the concentration of GSH in the neoplastic tissue will be much lower than normal at the time point when GSH concentration in healthy tissues is normal or even higher than normal. These findings could increase, when applied in cancer chemotherapy, both the safety and the effectiveness of the treatment.

Proposed Course:

Characterize in variety of tumor systems the effect of polar chemical compounds (such as DMSO and polymethylene bisacetamides) and antitumor agents on gene expressions which are associated with the malignant state.

Publications:

1. Wirth, P. J. and Thorgeirsson, S. S.: Glutathione Synthesis and Degradation in Fetal and Adult Rat Liver and Novikoff Hepatoma. Cancer Res. in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06137-02 LCHP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Mechanism of Chemically Induced Hepatomas and Ovarian Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. S. Thorgeirsson	Head, Biochemical Pharm. Sec.	LCHP NCI
OTHER:	S. Sakai	Visiting Fellow	LCHP NCI
	R. W. Downs	Clinical Associate	MD NIAMD

COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Chemical Pharmacology

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Biochemical Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to study the mechanism of chemically induced hepatomas and ovarian tumors in rodents and non-human primates. We are particularly interested in the role of environmental contaminants such as the polycyclic hydrocarbons, nitroso-compounds and N-acetylarylamines in initiating these tumors, as well as the role of genetic predisposition in development of these tumors. These tumors, at various time points during their development, will then be used as models for evaluating chemotherapeutic agents and other drugs which might be of therapeutic value. Topics of present interest are: (1) the role of metabolic activation, as well as the genetic control of activating enzymes, in the chemical initiation of hepatoma and ovarian tumors; (2) the time course of hepatoma and ovarian tumor initiation with particular emphasis on the changing enzyme patterns as the cells become increasingly dedifferentiated; (3) the role of nucleophilic agents such as N-acetylcysteine, L-cysteine and cysteamine in preventing the chemical initiation of hepatomas and ovarian tumors; (4) the characterization of cAMP and the β-receptor involvement in the chemical induction of hepatomas as well as the possible use of β-blockers and/or β-agonists in preventing the occurance of these hepatomas.

Project Description:

Objectives:

The main objectives of this project are: (1) to study in rodents and non-human primates the relationship between the activity, both basal and induced, of the enzymes which metabolically activate compounds such as polycyclic hydrocarbons, N-acetylarylamines, and nitroso-compounds, and toxicity as well as carcinogenicity of these compounds. The main emphasis will be focused on the liver, the ovary and the GI tract. The importance of these studies with respect to man is highlighted by reports indicating that inducibility of aryl hydrocarbon hydroxylase in cultured peripheral lymphocytes is correlated with the occurance of bronchogenic carcinoma. Furthermore, the 3-4 fold increase in ovarian cancer over the last 40 years in the USA, with the highest incidence in urban industrialized areas, indicate that part of this increase may be caused by environmental contaminants; (2) to clarify and define the role of nucleophilic agents such as N-acetylcysteine, L-cysteine and cysteamine in protecting against toxicity and carcinogenicity of antitumor agents and chemical carcinogens; (3) to study the time course of hepatoma and ovarian cancer initiation. The main emphasis will be on the changing enzyme patterns as the cells become increasingly de-differentiated, and the effect of established antitumor agents as well as other compounds of therapeutic interest on the development of these tumors; (4) to examine the role of cAMP and the β-receptor in the chemical initiation of hepatomas.

Methods Employed:

The principal methods employed are: (1) recording spectrophotometry of turbid solutions, (2) enzyme assays involving radiometric, spectrophotometric, and spectrophotofluorometric determinations of product formation or substrate disappearance, (3) radioisotopic measurements using tritium and carbon-14, (4) differential centrifugation, (5) polyacrylamide gel electrophoresis, (6) column and thin-layer chromatography, (7) high pressure liquid chromatography, and (8) bacterial culture technique.

Major Findings:

1. Induction of content and activity of hepatic and lung cytochrome P-450-dependent monooxygenases by 3-methylcholanthrene (MCA) was studied in male rhesus monkeys. Hepatic microsomes were concomitantly studied for mutagenic activation of N-2-acetylaminofluorene (AAF) as well as with the use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Hepatic N-hydroxylation of AAF was increased sixfold to sevenfold after MCA treatment; aryl hydrocarbon hydroxylase was similarly increased in both liver and lung. The hepatic cytochrome P-450 content was increased by 50%, and the Soret maximum in the CO-hemoprotein complex was shifted to the blue by 2 nm after MCA induction. Electrophoresis of hepatic microsomes from MCA-treated monkeys showed an increase in a polypeptide band of 54,000 molecular weight. Mutagenic activation of AAF by liver microsomes from untreated rhesus monkeys was low but was increased 40-fold after MCA treatment. No effect was observed on the mutagenic activation of N-hydroxy-2-acetylaminofluorene by liver microsomes after MCA treatment. Rhesus monkeys may provide a model for evaluation of the role of polycyclic hydrocarbon induction in chemically caused cancer and toxicity in primates.

2. Rat and mouse gonadal aryl hydrocarbon hydroxylase (AHH) activity was measured and characterized in control and MCA-treated animals. The ovarian AHH activity increased 2- to 3-fold in Sprague-Dawley, Lewis, and Brown-Norway rats following MCA treatment. The same treatment decreased the testicular AHH activity in Sprague-Dawley rats. The induction of AHH activity after MCA administration was the same in ovaries from weanling and adult Sprague-Dawley rats. Ovarian AHH activity was located in the microsomal fraction, required NADPH, and had a broad pH optimum between 7.0 and 8.8. Both carbon monoxide and antibody to cytochrome c reductase inhibited the ovarian AHH activity, suggesting that the hydroxylase is a cytochrome P-450-dependent monooxygenase.

Basal testicular and ovarian AHH activity was similar in C57BL/6N (B6) and DBA/2N (D2) mice; however, after MCA treatment an increase in gonadal AHH activity was observed only in B6 mice. Treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin resulted in an increase in testicular AHH activity in both B6 and D2 mice.

The oocyte toxicity of MCA in mice and rats was different. Seven days after a single i.p. injection of MCA, (80 mg/kg), 87% of the primordial oocytes were destroyed in the ovaries of B6 mice, 69% were destroyed in D2 mice, and none were destroyed in Sprague-Dawley rats. These data suggest that the oocyte toxicity of MCA in the mouse is related to the ovarian AHH activity, whereas no association is found between the ovarian AHH activity and oocyte toxicity in the rat.

The role of metabolism and sulfhydryl compounds in modifying 7,12-dimethylbenz(a)anthracene (DMBA) toxicity in D2 and B6 mice was studied. Treatment of B6 mice with polycyclic aromatic hydrocarbons (PAH) results in induction of the microsomal monooxygenases(s) while no change in monooxygenase(s) activity is found in D2 mice. Both strains are inducible by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Survival curves after single i.p. doses of DMBA were significantly different ($p < 0.05$) between these two strains. At 80 mg kg^{-1} 50% of the B6 mice were dead by 10 days and 95% at 12 days, whereas 95% of the D2 mice were alive at 35 days when the experiment was terminated. Treatment with TCDD resulted in no change in the survival curves of B6 mice after DMBA administration while the survival curves of D2 mice were similar to those of B6 mice. Treatment of B6 and TCDD induced D2 mice with α-naththoflavone (ANF) a known inhibitor of cytochrome P_1-450 (P-448) dependent oxidation, shifted the survival curves to the right and death occurred only after cessation of ANF treatment. Treatment of the mice with cysteamine and N-acetylcysteine gave no protection against DMBA lethality.

Similar effects of inducers, ANF, cysteamine and N-acetylcysteine on DMBA induced ovotoxicity were observed in both B6 and D2 mice. It is concluded that metabolic activation of DMBA results in both specific organ toxicity and death, neither of which can be prevented by administration of nucleophiles such as cysteamine and N-acetylcysteine.

We have proposed, on the basis that the rodent ovary contains an enzyme system(s) capable of metabolizing polycyclic aromatic hydrocarbons to reactive electrophilic intermediates known to cause cytotoxicity, mutation, and cancer, that if the human ovary contains similar enzyme systems, metabolic activation of environmental chemicals could explain the earlier menopause in cigarette smokers and the higher incidence of ovarian cancer in industrialized areas.

3. Preliminary experiments involving the expression of inducibility of cytochrome P-450 dependent monooxygenase by polycyclic hydrocarbons have been conducted in rat liver at various time points during chemically caused liver cancer. The results indicate that induction of C- and N-hydroxylations are differentially affected during the course of hepatic tumor initiation and promotion, and that the synthesis of selective forms of cytochrome P-450 is decreased.

4. Our first phase in the study on the relationship between catecholamine stimulated adenyl cyclase and β-receptor concentration in rat hepatocytes during *in vivo* exposure to the hepatocarcinogen 2-acetylaminofluorene (AAF) is now finished. The catecholamine stimulation of cAMP is normally observed *only* during fetal life and the early neonatal period. We have found that during the first 12 weeks of feeding AAF both catecholamine stimulated cAMP and the concentration of β-receptor in the plasma membrane are increased. This finding opens the possibility that the β-receptor may be an important "regulator" in chemically induced hepatic cancer.

Significant to Biomedical Research and the Program of the Institute:

Our studies are aimed at providing a better understanding of tumor initiation as well as tumor progression. This information will then be used to design chemotherapeutic regimens as well as new chemotherapeutic agents for treating these and other tumors.

Proposed Course of Project:

Continue the course outlined under Objective.

Publications:

1. Thorgeirsson, S. S., Sakai, S., and Adamson, R. H.: Induction of Monooxygenases in Rhesus Monkeys by 3-Methylcholanthrene: Metabolism and Mutagenic Activation of 2-acetylaminofluorene and Benzo[a]pyrene. *J. Natl. Cancer Inst.*, 60, 365-369, 1978.

2. Thorgeirsson, S. S., and Wirth, P. J.: Genetic Aspects of Metabolic Processing of Chemical Substances. In press, J. Toxicol. Environ. Health, 1978.

3. Nelson, W. L., Thorgeirsson, S. S., and Wirth, P. J.: Applications of the High Performance Liquid Chromatographic Techniques to Metabolic Problems of Oxidation on Nitrogen in Organic Molecules. In 2nd International Symposium on N-oxidation. London, Sept. 19-23, 1977. In press, 1978.

4. Mattison, D. R., and Thorgeirsson, S. S.: Gonadal Aryl Hydrocarbon Hydroxylase: Characterization and Response to Polycyclic Hydrocarbon in Mice and Rat. Cancer Res., 38, 1368-1373, 1978.

5. Mattison, D. R., and Thorgeirsson, S. S.: Smoking, Industrial Pollution, Menopause and Ovarian Cancer. Lancet, vol. 1, No. 8057, pp 187-189, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06138-02 LCHP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Mutagenicity and Carcinogenicity of Antitumor Agents and Chemical Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	None

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to evaluate the relationship between chemically induced mutagenesis and carcinogenesis. The primary emphasis is placed on antitumor agents, known environmental contaminants and model chemical carcinogens. A sensitive in vitro microbial test system is used for the detection of chemical mutagens. The research is directed at present to determine: (1) the role, if any, and the mechanism of metabolic activation in the mutagenesis caused by antitumor agents and chemical carcinogens; (2) the predictive value of the in vitro mutagenesis test for in vivo carcinogenesis; (3) the possible advantage and clinical applications of using the mutagenesis test to detect antitumor agents and other reactive compounds in various body fluids; (4) the effect of structural modification on the mutagenicity of antitumor agents and model chemical carcinogens; (4) the relationship between the in vivo and in vitro protective effects of nucleophiles, such as L-cysteine, N-acetyl-cysteine and cysteamine, against toxicity of antitumor agents.

Project Description:

Objectives:

The main objectives of this project are: (1) to examine the role of both microsomes and nuclear membranes from rodent hepatocytes in activating procarcinogens to ultimate carcinogens and/or mutagens; (2) to study the relationship between mutagenicity in the in vitro microbial test system (Salmonella test system) and carcinogenicity of antitumor agents and chemical carcinogens; (3) to examine the possible advantage and clinical applications of using the mutagenesis test to detect antitumor agents and other reactive compounds in body fluids; (4) to study the possible role of nucleophiles in protecting against toxicity of antitumor agents.

Methods Employed:

The principal methods employed are: (1) bacterial culture technqiue, (2) differential centrifugation, (3) enzyme assays, (4) recording spectrophotometry, and (5) high pressure liquid chromatography.

Major Findings

1. We have used the Salmonella test system, originally developed by B. N. Ames et al., as a means of detecting the mutagenic effects of chemical compounds. This system is based on backward mutation in a specific locus of the histidine operon in a histidine dependent Salmonella typhimurium mutant resulting in histidine independence. In the course of our studies with this system, we developed a forward mutation assay with the histidine independent Salmonella mutant by selecting for 8-azaguanine resistance due to mutation in the structural gene for hypoxanthine-guanine phosphoribosyl transferase (HGPRT). With this system we are now capable of examining both the frequency of the specific backward mutation as well as the general forward mutation (presumably detecting the entire set of possible mutagenic lesions in DNA caused by chemical compounds), At the present time several antitumor agents are being examined in this system. Considerable time and effort has been spent in demonstrating and teaching the mutation assay to researchers both within our own division and other divisions of NCI.

2. The bacterial mutagenesis system was used to evaluate the role of the deacetylase and sulfotransferase in the mutagenic activation of N-hydroxy-2-acetylaminofluorene. The mutagenicity of N-hydroxy-2-acetylaminofluorene mediated by liver or kidney microsomes was independent of treatment of mice or rats with microsomal enzyme inducers and, at equivalent protein concentrations, was the same whether mouse liver or mouse kidney microsomes were used. N-Hydroxy-2-acetylaminofluorene mutagenesis with mouse and rat liver or kidney microsomes was completely inhibited by 0.1 mM paraoxon and partially inhibited by addition of 3'-phosphoadenosine 5'-phosphosulfate when rat liver 9000 x g supernatant was used. Although the rates of deacetylation of 2-acetylaminofluorene and N-hydroxy-2-acetylaminofluorene in vitro were

lower in kidney microsomes than in liver microsomes of either the mouse or the rat, both reactions were also completely inhibited by 0.1 mM paraoxon. The addition of ascorbic acid (10 mM) approximately doubled the mutagenicity of N-hydroxy-2-acetylaminofluorene in the presence of mouse kidney microsomes but had no effect on the mutagenicity of N-hydroxy-2-aminofluorene in the absence of microsomal protein. Butylated hydroxytoluene (10 mM) had very little or no effect, in either the absence or presence of 10 mM ascorbic acid, on the mutagenicity of N-hydroxy-2-acetylaminofluorene with liver or kidney microsomes. The data indicate that deacetylation is the most important step in the mutagenic activation of N-hydroxy-2-acetylaminofluorene by mouse and rat liver and kidney fractions and that the arylnitrenium ion, rather than the nitroxyl free radical, is the electrophilic species interacting with the bacterial DNA, resulting in the frameshift mutation.

3. The mechanism of in vitro mutagenic activation of N-hydroxy-2-acetylaminofluorene (N-OH-AAF) in the bacterial test system by isolated rat and mouse liver cell nuclei as well as the in vitro covalent binding of N-OH-AAF to nuclear nucleic acids and proteins were also studied. Mutagenic activation of N-OH-AAF and 2-acetylaminofluorene by liver cell nuclei from both rat and mouse was observed. The mutagenic activation of N-OH-AAF by the mouse nuclei was 6-7 times greater than in the rat nuclei (2300 vs 350 revertants per 10 μg N-OH-AAF). The mutagenicity of N-OH-AAF was increased two fold when NADPH was included with the nuclei in the assay mixture, and this increase in N-OH-AAF mutagenicity was inhibited by α-naphthoflavone. The carboxyesterase/amidase inhibitor paraoxon (10^{-5} M) completely inhibited the mutagenic activation of N-OH-AAF in both mouse and rat liver nuclei. Paraoxon (10^{-4} M) had no effect on the mutagenic activation of N-OH-AAF in the 100,000 x g supernatant fraction from rat liver, whereas fifty percent inhibition of N-OH-AAF mutagenesis was observed when adenosine-3'-phosphate-5'phosphosulfate (PAPS) was added to the supernatant fraction. The addition of rat liver 100,000 x g supernatant to mouse nuclei resulted in decreased mutagenicity of N-OH-AAF which could be further inhibited by paraoxon (10^{-5} M). On the other hand the addition of rat liver 100,000 x g supernatnant to rat liver nuclei resulted in an increase in N-OH-AAF mutagenicity which was insensitive to inhibition by paraoxon. Antioxidants such as vitamin E and butylated hydroxytoluene (BHT) at 10^{-2} M concentration inhibited the mutagenic activation of N-OH-AAF by 20-30 percent in the mouse nuclear preparation whereas vitamin C at the same concentration increased the N-OH-AAF mutagenesis 2-3 fold. Addition of cysteamine and methionine (10^{-2} M) to the mouse nuclear preparation resulted in 60 and 30 percent inhibition, respectively, of N-OH-AAF mutagensis.

The in vitro covalent binding of N-OH-AAF to nuclear nucleic acids and proteins was observed with both rat and mouse nuclei. The degree of covalent binding to nuclear protein and RNA was similar in nuclei from both species, but the binding to DNA in the rat nuclei was twice that observed in mouse DNA (21 vs. 10 pmol mg^{-1} 10 min^{-1}). Paraoxon (10^{-4}M) inhibited by more than 70 percent the covalent binding of N-OH-AAF to nuclear nucleic acid and proteins in both mouse and rat nuclei. Similar inhibition by paraoxon of the covalent binding of N-OH-AAF to microsomal protein was observed, whereas paraoxon had no effect on the binding of N-OH-AAF to protein in 100,000 x g supernatant fraction from rat liver.

Combining rat liver nuclei with the 100,000 x g supernatant had no significant effect on the covalent binding of N-OH-AAF to nuclear nucleic acid and protein except when PAPS was included, which resulted in a significant ($P < 0.05$) decrease in the binding to nuclear RNA and proteins. Cysteamine and vitamin C (10^{-2}M) significantly ($p < 0.05$) inhibited the covalent binding of N-OH-AAF to nuclear RNA and protein, whereas methionine at the same concentration had no effect.

It is concluded that the first step in the *in vitro* mutagenic activation of N-OH-AAF by isolated rat and mouse liver cell nuclei and/or the 100,000 x g supernatant fraction from rat liver, as well as the *in vitro* covalent binding to nuclear nucleic acids and protein, occurs primarily via deacetylation by either the membrane bound amidase or by the N-O-acyltransferase in the supernatant fraction.

4. Several biochemical parameters associated with *in vivo* 2-acetylaminofluorene (AAF) carcinogenesis and *in vitro* AAF mutagenesis in the *Salmonella* test system were evaluated in subcellular liver fractions of the Cotton rat, a species resistant to AAF carcinogenesis. Pretreatment with 3-methylcholanthrene (MC) induced a) microsomal cytochrome P-448, b) microsomal polypeptides of approximately 50,000 and 54,000 daltons, c) AAF *N*-hydroxylase (together with other AAF hydroxylases), and d) *in vitro* AAF mutagenicity mediated by microsomes. Aryl hydrocarbon hydroxylase (EC 1.14.14.2), however, was unaffected by MC-pretreatment. Low concentrations (1μg/plate) of AAF or *N*-hydroxy-AAF were highly mutagenic (>1000 colonies/plate) in the presence of low concentrations (0.4 mg/plate) of microsomal protein. Similar to the situation in both the mouse and Sprague-Dawley rat, deacetylation and mutagenicity of both AAF and *N*-hydroxy-AAF were partially inhibited by 0.001-0.1 μM paraoxon and completely inhibited by 100 μM paraoxon, indicating the involvement of a microsomal deacetylase in the mutagenic activation of AAF and *N*-hydroxy-AAF. The activity of *N*-hydroxy-AAF sulfotransferase in Cotton rat liver was one-third that of the Sprague-Dawley rat and caused a decrease (45%) in cytosol mediated *N*-hydroxy-AAF mutagenesis, indicating that this enzyme is not important in the mutagenic activation of N-hydroxy-AAF.

AAF mutagenesis with microsomes from untreated animals was much more sensitive to inhibition by α-naphthoflavone (ANF, 1-10 μM) than that with microsomes from MC-treated animals and at 50 μM ANF this process was completely inhibited in both cases. Similarly, AAF N-hydroxylase from untreated animals exhibited a greater sensitivity to ANF (1-10 μM) than that of MC-treated animals, but the difference was much less pronounced, and at higher ANF concentrations (500 μM) this difference was reversed with the enzyme from MC-treated animals showing the greater inhibition (90% *vs* 60%). Inhibition of aryl hydrocarbon hydroxylase required ANF concentrations greater than 10 μM and the enzyme from MC-treated animals was more sensitive to this agent (60% inhibition) than that of untreated animals (35% inhibition). The effect of ANF on AAF ring hydroxylations by microsomes from untreated and MC-treated animals was quite variable with wide ranges of stimulation (0-100%) and inhibition (0-52%) at various ANF concentrations (1-500 μM), indicating that more than one cytochrome P-450 (or P-448) dependent monooxygenase catalyzes these reactions.

The Cotton rat appears to possess all the known enzyme systems to activate AAF into an ultimate carcinogen or mutagen. Exploration of other biochemical properties, such as interaction of AAF with subcellular macromolecules or *in vivo* AAF metabolism may provide further insight into the resistance of this species to AAF carcinogenesis.

5. Hydrazine compounds, including procarbazine, are not mutagenic in the classic Salmonella test system (i.e., backward mutation) when activating enzymes from mouse, rat or rhesus monkey were used. Further studies on the mutagenicity of hydrazines are being conducted in the forward mutagenesis test system.

6. The Salmonella typhimurium system was used to test the mutagenic activity in urine from rat, mouse and guinea pig after administration of the potent urinary bladder carcinogen N-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide (FANFT). High mutagenic activity was found in urine from mouse and rat, species known to be susceptible to the carcinogenic effect of FANFT. In contrast, no mutagenic activity was found in urine from guinea pig, which is resistant to FANFT. It is concluded that the Salmonella test system may be of value in detecting urinary mutagens and potential carcinogens as well as differentiating between individuals who are susceptible or resistant to urinary tract carcinogens. These studies have been extended to include the examination of the mutagenic activity of: plasma and urine in patients on chemotherapy, urine from people living in areas with high incidence of bladder carcinoma (Egypt), and in extracts from transplantable tumors after treatment with antitumor agents known to be active or inactive against these tumors.

7. The lethality of cyclophosphamide and melphalan is significantly reduced in mice (both C57B6/6N) and/or DBA/2N) by pretreatment with methionine and cysteamine, respectively. However, no apparent reduction in mutagenic potential of these antitumor agents were observed in the Salmonella test system when the nucleophiles were included.

Significance to Biomedical Research and the Program of the Institute

Our studies are aimed at providing better understanding of the mechanism of toxicity, mutagenicity and potential carcinogenicity caused by antitumor agents and chemical carcinogens. The information derived from these studies may provide a sounder base for designing chemotherapeutic regimes, early identification of individuals at risk to develop cancer, and could provide means to prevent toxic side effects of antitumor agents.

Proposed Course of Project:

Continue the course outlined under Objectives and Major Findings.

Publications:

1. Thorgeirsson, S. S., Wirth, P. J., Nelson, W. L., and Lambert, G. H.: Genetic Regulation of Metabolism and Mutagenicity of 2-acetylaminofluorene and Related Compounds in Mice, pp 869-886. In Watson, J.D., and Hiatt, H. (eds.): Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, 1977.

2. Thorgeirsson, S. S., Wirth, P. J., and Nelson, W. L.: Metabolism and Mutagenicity of 2-acetylaminofluorenes and Related Compounds, pp 575-580. In Ullrich, V., Hildebrandt, A. G., Estabrook, R. W., and Conney, A. H. (eds.): Microsomes and Drug Oxidations. Oxford, Pergamon Press, 1977.

3. Thorgeirsson, S. S.: Mutagenic Activation of N-acetylarylamines. In Perugia Quadrennial Conference on Tumors of Early Life in Man and Animals, in press, 1978.

4. Schut, H. A. J., Wirth, P. J., and Thorgeirsson, S. S.: Mutagenic activation of N-hydroxy-2-acetylaminofluorene in the Salmonella test system. The role of deacetylation by liver and kidney fractions from mouse and rat. Mol. Pharmacol., in press, 1978.

5. Schut, H. A. J., and Thorgeirsson, S. S.: In Vitro metabolism and mutagenic activation of 2-acetylaminofluorene and N-hydroxy-2-acetylaminofluorene by Cotton rat subcellular fractions. In press, Cancer Res., 1978.

6. Sakai, S., Reinhold, C. E., Wirth, P. J., and Thorgeirsson, S. S.: The mechanism of in vitro mutagenic activation and covalent binding of N-hydroxy-2-acetylaminofluorene in isolated liver cell nuclei from rat and mouse. In press, Cancer Res., 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06141-01 LCHP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Kinetics and Pharmacologic Effects of Polymer Linked Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. S. Zaharko	Head, Pharmacokin. & Pharmacody. Sec.	LCHP	NCI
OTHER:	R. H. Adamson	Chief	LCHP	NCI
	M. Przybylski	Asst. Prof. Johannes Gutenberg Universitat Visiting Fellow, 1977-78		
	H. Ringsdorf	Prof. Johannes Gutenberg Universitat		
	M. Chirigos	Head, Virus & Disease Modification Sec.	LVRN	NCI
	W. P. Fung	Staff Fellow (appt. ended April 1978)	LCHP	NCI
	L. M. Ramonas	Staff Fellow (appt. began April 1978)	LCHP	NCI

COOPERATING UNITS (if any)

Organic Chemistry Department, Johannes Gutenberg Universitat, Mainz, West Germany and Virus and Disease Modification Section, NCI

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

NCI, NIH, Bethesda, Maryland 20014

INSTITUTE AND LOCATION

Pharmacokinetics and Pharmacodynamics Section

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.25	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline

The biological effects and pharmacokinetics of synthetic polymers linked to antitumor agents are being investigated. Initial trials are being conducted with divinylethermaleic anhydride copolymer covalently bound to methotrexate. Other polymers and antitumor agents are to be studied.

PHS-6040

Project Description:

Objectives:

The purpose of the project is to investigate the pharmacology of antitumor agents linked covalently to synthetic polymers. The rationale behind the work is two fold. First, the pharmacokinetics of a polymer-linked antitumor agent should be different from that of the agent alone. This may alter selective toxicity as illustrated by the work of DeDuve and Trouet (1972) with adriamycin-DNA complexes. The advantage of using synthetic rather than natural polymers is the ability to control more closely their chemical composition and hence reproducibility. Second, many synthetic polymers are immune stimulating agents, whereas most cytotoxic agents have immunosuppressive effects. Thus it is postulated that an enhanced antitumor effect could be obtained by administering a polymer-linked agent. This effect could arise because a lesser degree of immunosuppression in the host would allow for a more effective action of the tumor antigens released following exposure to cytotoxic antitumor agents.

Method and Major Findings:

The antitumor agent methotrexate, (MTX) was selected for initial testing of these hypotheses because of our experience with this drug and the background information we have gathered for comparative purposes. The synthetic polymer chosen was divinylethermaleic anhydride copolymer (DIVEMA, Pyran). It is known to modulate the host's immune response and possesses antitumor activity alone against certain tumor systems.

The DIVEMA-MTX copolymer was evaluated against the L1210 ascites tumor as a system which is sensitive to MTX but not to DIVEMA alone. Preliminary results indicate an antitumor effect (ILS 200%) for DIVEMA-MTX greater than for MTX alone at optimal dose and schedule, or for equivalent doses of MTX plus DIVEMA.

The DIVEMA-MTX copolymer was also tested for activity against the Lewis Lung subcutaneous tumor as a system which is insensitive to MTX but sensitive to DIVEMA alone. The DIVEMA-MTX copolymer prolonged the life span of these mice by 40% over controls. At equivalent doses and the same schedules, MTX, DIVEMA, or DIVEMA plus MTX were ineffective. At higher doses, DIVEMA alone was effective in prolonging survival time, whereas higher doses of the MTX-DIVEMA polymer were toxic. This toxicity was a consequence of an alteration in MTX pharmacokinetics, resulting in prolonged plasma concentrations of MTX, when this drug was administered bound to the polymer.

The DIVEMA-MTX copolymer is provided as an anhydride which is insoluble in aqueous media and biologically inactive until the anhydride groups hydrolyse to carboxylic groups. We formulated this copolymer for *in vivo* injection by dissolving it in the weak base ($NaHCO_3$). In addition to hydrolysis of the carboxylic groups, it appears that a neighboring group effect is also causing MTX to dissociate rapidly from the polymer. However the *in vivo* DIVEMA-MTX effect is dramatically different from the DIVEMA plus MTX effect. We are currently studying this in vitro disassociation of MTX from the polymer to determine its extent and rate. Dr. Ringsdorf and his group are considering the possibility of additional synthetic linkages of MTX to the polymer since our results suggest we may be getting the in vivo effects with only a small portion of the polymer-MTX complex.

In cooperation with the Virus and Disease Modification Section, NCI, studies were conducted to determine the immune function of the DIVEMA-MTX copolymer. We were concerned that linking MTX to DIVEMA covalently would reduce or eliminate the known immune adjuvant activity of DIVEMA. Two tests (a tumor cell vaccine protective test and a macrophage activation test) indicated that the DIVEMA-MTX polymer retains the majority of the DIVEMA's immune adjuvant activity.

Studies will continue both in vitro and in vivo with DIVEMA linked to MTX as well as to other antitumor agents in attempts to understand the pharmacology of such polymer complexes.

Publications:

1. Zaharko, D. S., Przybylski, M., and Oliverio, V. T.: "Binding of of Anticancer Drugs to Carrier Molecules. In DeVita, V. T. and Busch, H. (eds.): Cancer Drug Development. New York. Academic Press. (In press) 1978.

2. Przybylski, M., Zaharko, D. S., Chirigos, M. A., Adamson, R. H., Schultz, R. M. and Ringsdorf, H.: DIVEMA-Methotrexate: Immune adjuvant role of polymeric carriers linked to antitumor agents, Cancer Treatment Reports (in press), 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06142-01 LCHP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Thymidine Pharmacology in Mice and Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. S. Zaharko	Head, Pharmacokin & Pharmacody. Sec. LCHP NCI
OTHER:	B. C. Giovanella	Senior Investigator, Stehlin Foundation for Cancer Research

COOPERATING UNITS (if any)

Stehlin Foundation, Cancer Research Laboratory, Houston, Texas

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Pharmacokinetics and Pharmacodynamics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An HPLC method for measuring thymidine and thymine in plasma and urine has been developed. Data is being collected to determine the pharmacokinetics of thymidine and thymine in mice and man following oral, i.p., i.v. routes of administration.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To investigate the pharmacokinetics of thymidine (TdR) in animals.

Methods and Major Findings:

Recently Giovanella (1977) has reported that TdR has antitumor effects when used at extremely high concentrations (10^{-3} M) in vitro on virally transformed cells, but not on their nontransformed counterparts. He also has reported similar antitumor effects in vivo with very large doses of TdR. A cooperative study with Dr. Giovanella is underway in which plasma concentrations of TdR are being measured in mice and man after TdR administration. The goal of this study is to determine whether it is possible to achieve TdR concentrations in man which have been found to have cytotoxic effects in vitro and antitumor effects in vivo in mice.

We have developed an HPLC method for measuring TdR and thymine (ThM) in plasma and urine. We are using this technique to collect data following single dose injections and during constant infusions of TdR in order to develop a pharmacokinetic model for TdR behavior in vivo.

Preliminary data indicate that the clearance of TdR in man is much more rapid than in mice, which is contrary to what would be expected on the basis of clearance being an inverse function of body weight. In addition the conversion of TdR to ThM is more rapid and the plasma ThM/TdR ratio is higher in humans than in mice. The blood of man is capable of converting TdR to thymine in vitro.

The feasibility of using TdR phosphorylase inhibitors to block the catabolism of TdR to thymine is being evaluated. Two potential phosphorylase inhibitors with relatively low toxicity (5-diazauridine and 5-allyl-2'-deoxyuridine) are under study. Another compound, 5-diazouracil does not appear to block TdR phosphorylase in mice, but prevents the further catabolism of ThM.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07100-05 LCHP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Tumor Biology and Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. L. Cysyk	Pharmacologist	LCHP	NCI
OTHER:	R. L. Magin	Biophysicist, IPA	LCHP	NCI
	R. H. Adamson	Chief	LCHP	NCI
	D. G. Johns	Chief	LMCB	NCI
	M. M. Chassin	Clinical Associate	LMCB	NCI

COOPERATING UNITS (if any)

Laboratory of Medicinal Chemistry and Biology, NCI

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Drug Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A microwave apparatus was designed and constructed to study the effect of localized hyperthermia on tumor growth, the biochemical alterations produced by hyperthermia, and the therapeutic effectiveness of combining chemotherapy with local hyperthermia. In a study to compare the anti-tumor properties of PALA (NSC-224131) in a spectrum of transplantable tumors in mice, PALA was found to be highly active against most solid tumors but to possess only minimal activity against several rapidly growing leukemias. The adenosine deaminase inhibitor, 2'-deoxycoformycin was found to potentiate the cytotoxic and antitumor activity of several adenosine analogues that are substrates for adenosine deaminase and that are inactivated by this enzyme. In addition, 2'-deoxycoformycin exhibited immunosuppressive activity in several experimental systems. A limited study was conducted comparing the potentiating effects of 2'-deoxycoformycin and EHNA on the cytotoxic properties of xylosyladenine.

Project Description

Objectives:

To investigate potential and established cancer chemotherapeutic agents with respect to their effect on experimental tumors; to determine the relationship between various biological properties of tumors and response to chemotherapy; to study structure-activity relationships of various antineoplastic agents; to develop underlying principles for successful cancer therapy; to determine the therapeutic effectiveness of rationally combined agents and rationally combined treatment modalities.

Methods Employed:

Usual biologic, pharmacologic and biochemical techniques including: tumor transplantation, enzymology, and tissue culture techniques. A microwave apparatus for localized hyperthermia was developed.

Major Findings:

Evaluation of Agents for Antitumor Activity

A. N-(Phosphonacetyl)-L-Aspartic Acid (PALA)

This rationally synthesized inhibitor of aspartate transcarbamylase was further evaluated for its activity against several murine leukemias and plasma cell tumors. PALA had little or no activity in all these systems except for minimal activity against leukemia P388 (40% increase in median survival time). However, PALA had good activity against several solid tumors including the B16 melanoma, Lewis lung carcinoma, and M5076 ovarian carcinoma, producing long term survivors in 20-70% of mice bearing these solid tumors. A Lewis lung carcinoma was made resistant to PALA for studies on mechanism(s) of resistance (see Annual Report Number Z01-CM-06108-09).

B. Studies with the Adenosine Deaminase Inhibitors 2'-Deoxycoformycin (2'-DCF) and Erythro-9-(2-Hydroxy-3-Nonyl)-Adenine (EHNA)

Adenosine and seven analogs were examined as substrates for P388 murine leukemia adenosine deaminase. The following compounds were substrates for the deaminase: adenosine, 8-aza-adenosine, 6-chloropurine ribonucleoside, 3'-deoxyadenosine, formycin A, 4'-thioadenosine, and 9-β-D-xylofuranosyladenine. Tubercidin was neither a substrate nor an inhibitor for the enzyme. Formycin A and 8-aza-adenosine were deaminated 18 and 12 times faster than adenosine, whereas all the other analogs were deaminated somewhat more slowly than adenosine. The deamination of all the analogs was inhibited by 2'-DCF, and the ID_{50} values using 2'-DCF as an inhibitor were similar for all the compounds ($\sim 10^{-10}$ M). 2'-DCF also potentiated the cytotoxic effects of all the analogs except tubercidin against leukemia P388 in vitro. 2'-DCF alone at levels of 1 μg/ml or 10 μg/ml had no cytotoxic effect. Both 2'-DCF and EHNA potentiated the antitumor activity of 9-β-D-xylofuranosyladenine (xylosyladenine) against leukemia P388. Xylosyladenine at the optimal daily dose of 50 mg/kg increased the median survival time of mice with leukemia P388 5 days over that of saline-treated controls. Mice treated with 2'-DCF or EHNA died at the same time as controls. In contrast, mice treated with 2'-DCF or EHNA 10 min prior to treatment with xylosyladenine at reduced optimal doses survived to 30 days (2'-DCF) or 38 days (EHNA), with 20 to 30% of the mice being long-term survivors and apparently cured of their leukemia.

The adenosine deaminase inhibitor 2'-DCF not only potentiates the cytotoxic and antitumor activity of various adenosine analogs, but it also exhibits immunosuppressive activity in several experimental systems. For example, guinea pigs were sensitized to mycobacterium and their delayed hypersensitivity skin test response to challenge with PPD was measured. Treatment with 2'-DCF either before sensitization or before recall signficantly diminished skin test response. In another system, monkeys were treated with 2'-DCF for a one week period. The response of lymphocytes from these animals, when tested in vitro for responsiveness to a non-specific mitogen, was diminished compared to control animal cells and to their own cells when tested pre-treatment. Also, immunosuppressive activity was found using an allograft tumor test system in which LSTRA cells (a Moloney virus-induced leukemia in BALB/c mice) were transplanted subcutaneously into C57Bl/6 mice across an H-2 histocompatibility barrier. Thus, allograft acceptance as evidenced by tumor death in the animals did not occur in untreated mice, in mice treated daily for 6 days after transplantation with EHNA or 2'-DCF, or in mice pretreated with EHNA. However, allograft acceptance occurred in mice pretreated with 2'-DCF 24 hr before tumor transplantation or with 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU; carmustine), a positive control. These studies support the concept that certain immunological functions can be suppressed in vivo by adenosine deaminase inhibitors just as they can be restored if adenosine deaminase is provided to animals deficient in this enzyme.

C. Effect of Cysteamine and N-Acetylcysteine on the Antitumor Activity of Nitrogen Mustard and Adriamycin

Two nucleophiles, cysteamine and N-acetylcysteine, are being evaluated for their protective effects against tumor induction by antitumor agents and other carcinogens. These nucleophiles have been used in humans for treatment of acetaminophen overdosage. It is obvious that these chemoprotective agents must be non-toxic and must also not reduce the therapeutic effects of antitumor drugs. In the experimental mouse plasma cell tumor YPC-1 neither cysteamine nor N-acetylcysteine reduced the antitumor effect of nitrogen mustard. In addition, the antitumor effect of adriamycin against mouse leukemia P388 was not affected by prior administration of either nucleophile.

D. Chemotherapy of Human Colon Carcinoma Xenografts in Athymic Nude Mice

Several new agents under development for clinical trial were examined for activity in the colon xenografts CX1, CX2, and CX3. These tumors failed to respond to a number of agents with a variety of mechanisms of action and which are active against murine tumors. These results indicate that the colon xenografts may be valid models for the human disease based on poor response to chemotherapy.

E. Development of Local Microwave Hyperthermia as a Treatment Modality

A microwave system was designed and constructed which provides simultaneous controlled, localized hyperthermia in the tumors of four experimental animals. The components of the system are a 2.45 GHz microwave source, a four-way power-dividing network with reflected power monitors, a temperature controlled microwave power regulator, and small direct contact microwave applicators. Adjustment of the temperature control results in elevated temperatures in the center of tumors which could be maintained to within ± 0.1°C without production of significant whole body hyperthermia. The temperatures at the edges of the locally heated tumors were found to vary within ± 1.0°C of the center temperature when the tumor was encapsulated in a bolus of muscle-equivalent dielectric material.

Six different subcutaneously implanted mouse tumors (B16 melanoma, Lewis lung carcinoma, Glioma 26, Ovarian carcinoma M5076, Colon adenocarcinoma, Mammary adenocarcinoma Line 16C) were tested. A single local hyperthermia treatment (43°C, 60 min) reduced the size and retarded the growth of each of the treated tumors compared with the controls. A difference in the sensitivity of the various tumors to local hyperthermic treatment was observed. Short growth delays (1-5 days) and moderate size reduction (20-50 percent) were observed in three of the treated tumors while longer growth delays, greater size reduction, and a few cures were seen in the remaining three: Colon 38 (13 days, 71 percent), Glioma 26 (7 days, 67 percent, 1 cure) and Ovarian M5076 (9 days, 71 percent, 1 cure). Three sequential treatments of the Colon 38 at approximately weekly intervals increased the growth delay to 19 days and the tumor size inhibition to 85 percent. Four sequential treatments of the Ovarian M5076 at three day intervals resulted in a 12 day growth delay and a tumor size inhibition of 87 percent. Cellular biochemical alterations produced by hyperthermia are being investigated for a rational approach to combining heat and specific drugs to exploit the heat effects.

Presently, local hyperthermia is being combined with a variety of drugs to determine which drug effects are enhanced by the heat treatment. Preliminary results indicate that local hyperthemia increases the tumorcidal action of adriamycin and bleomycin. Analysis of the treatment sequence of drug and heat and the mechanism of thermal drug enhancement will be pursued with particular emphasis on the effect of heat on uptake of drug by the tumor and on the reactivity of the drug at its site of action. Representative drugs of the various classes of antitumor agents (alkylating agents, antimetabolites, etc.) will be combined with local hyperthermia and the biochemical effects determined. The combination of local hyperthermia and drugs is an especially appealing combination, since local hyperthermia has little or no systemic toxicity.

Significance to Biomedical Research and the Program of the Institute

Work on this project is directed towards development of new drugs and other modalities for the treatment of cancer and towards gaining a better understanding of the principles of effective chemotherapy. The development of PALA and the adenosine deaminase inhibitors for clinical trial is based in part on investigations within this project and collaborative research with other investigators.

Proposed Course of Project

During the next year some new antitumor agents will be examined for their effectiveness against various experimental tumors. Other nucleophiles will be examined for their effects on the antitumor activity of various alkylating agents and their ability to protect against the adverse effects of the alkylating agents. The effectiveness of microwave hyperthermia as a treatment modality in conjuction with various chemotherapeutic agents will be evaluated in a spectrum of animal tumor systems.

Publications

1. Adamson, R. H., Chassin, M. M., Chirigos, M. A., and Johns, D. G.: Mode of Action. Some aspects of the pharmacology of the adenosine deaminase inhibitors 2'-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl)-adenine. Current Chemotherapy II: 1116-1118, American Society for Microbiology, 1978.

2. Chassin, M. M., Adamson, R. H., and Johns, D. G.: An enzyme inhibition assay for 2'-deoxycoformycin and its application to the study of the relationship between drug concentration and tissue adenosine deaminase in the dog. Biochem. Pharmacol. (in press), 1978.

3. Johns, D. G., Wolpert-Defilippes, M. K., Mandelbaum-Shavit, F., Chabner, B. A., Bono, V. H. Jr., and Adamson, R. H.: Pharmacological action of maytansine. Current Chemotherapy II: 1180-1182, American Society for Microbiology, 1978.

4. Johnson, R. K., Swyryd, E. A., and Stark, G. R.: Effects of N-(phosphonacetyl)-L-aspartate on murine tumors and normal tissues *in vivo* and *in vitro* and the relationship of sensitivity to rate of proliferation and level of aspartate transcarbamylase. *Cancer Res.* 38: 371-378, 1978.

5. Chassin, M. M., Chirigos, M. A., Johns, D. G., and Adamson, R. H.: Adenosine deaminase inhibition for immunosuppression. *N. Engl. J. Med.* 296: 1232, 1977.

6. Johns, D. G., Sieber, S. M., and Adamson, R. H.: Studies on the antitumor activity of tilorone. In Chirigos, M. A. (Ed.): *Modulations of Host Immune Resistance in the Prevention or Treatment of Induced Tumors*. Washington, D. C., DHEW Publications No. (NIH) 77-893, 1977, pp. 117-121.

7. Magin, R. L., and Kantor, G.: Comparison of the heating patterns of small microwave (2450 MHz) applicators. *J. of Bioengineering* 1 #516: 493-509, 1977.

8. Mimnaugh, E. G., Waring, R. W., Sikic, B. I., Magin, R. L., Drew, R., Litterest, C. L., Gram, T. E., and Guarino, A. M.: Effect of whole-body hyperthermia on the disposition and metabolism of adriamycin in rabbits. *Cancer Res.* 38: 1420-1425, 1978.

SUMMARY REPORT OF THE LABORATORY OF MOLECULAR PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1977 to September 30, 1978

This laboratory is engaged in studies which approach the cancer chemotherapy problem at the level of molecular and cellular biology. The emphasis currently is on anti-tumor agents which directly attack DNA, particularly alkylating agents, nitrosoureas, platinum complexes and ionizing radiation, as well as DNA intercalating agents such as adriamycin, ellipticine and actinomycin. These studies have been facilitated by new methods developed in this laboratory which make it possible to measure DNA damage in cells treated with pharmacologic doses of these agents.

The first objective, which again was the major area of investigation during the past year, is to define the types of DNA damage which can be measured using the new filter elution techniques. An integral part of this problem is to investigate the mechanisms of the filter elution phenomena and to develop new variations of technique. The following types of DNA lesions had been shown to be detectable by these methods: single-strand breaks, alkali-labile sites, DNA-protein crosslinks and DNA interstrand crosslinks. These studies relate to drug treatment of mammalian cells in culture.

The efforts of the past year have focused on the quantitation and mechanism of detection of the 2 types of crosslinks and on modifications of technique to permit studies of DNA double-strand breaks and of drug effects on DNA replication.

The DNA crosslinking aspect was selected for intensive study because of the likelihood that the bifunctional agents, including most alkylating agents, chloroethylnitrosoureas, and platinum complexes, exert their major cytotoxic and antitumor actions by producing this type of effect. This concept was supported last year by our finding that the sensitivities of 3 human colon tumor lines to MeCCNU correlated with the extent of DNA crosslinking as measured by the direct alkaline elution assay. Included in this measurement however are both DNA-protein crosslinks and DNA interstrand crosslinks. A major problem is to establish the theoretical and methodologic foundations for the separate quantitation of these 2 types of DNA damage.

A significant advance during the past year has been in the area of DNA-protein crosslinks. The physical basis of this measurement has now been shown to be the adsorption of protein to the filters in the alkaline elution assays. By minor modifications of procedure it was possible to clearly separate the DNA single-strands linked to protein from those that are not linked to protein. This permitted the development and testing of a simple mathematical model from which the DNA-protein crosslink frequency could be calculated. The testing of this theory required a compound that would generate DNA-protein crosslinks in cells without at the same time producing interstrand crosslinks or strand breaks. We found that trans-Pt(II)diamminedichloride, although inactive as an antitumor agent, met the requirement, and we succeeded in using it to validate the theory.

The biological significance of DNA-protein crosslinks has in the past been difficult to evaluate because of technical limitations. As a result of this work, it is now possible to quantitate DNA-protein crosslink frequencies produced by pharmacologically relevant doses of various agents.

This advance was immediately applicable to a problem concerning DNA intercalators. Early in the current year, we tested adriamycin by the alkaline elution technique because others had reported that the drug generates DNA single-strand breaks. We found to our surprise that, although the standard alkaline sedimentation technique confirmed the production of breaks, alkaline elution showed no trace of them. This result was unprecedented in that numerous agents, found by standard techniques to produce either frank breaks or alkali-labile lesions, never before failed to show the expected effects in the alkaline elution assay. However, when adriamycin-treated cells were assayed by the proteinase method (in which the lysed cells are treated with this enzyme prior to alkaline elution), the expected frequency of single-strand breaks became apparent. This immediately suggested that, in addition to breaks, the drug was inducing the formation of DNA-protein crosslinks. The evidence further suggested that the DNA-protein links were localized at the break sites. We hypothesized that the DNA-protein links and strand breaks are due to a single event in which the drug directly or indirectly causes an endonuclease to produce a single-strand break and that the enzyme remains covalently bound to one terminus of the break. This dual effect had previously been described in the action of nicking-closing enzymes (DNA relaxase) which act on supercoiled DNA. It seemed possible that this type of effect was responsible for our observation because of the known effect of intercalation on DNA coiling. To test this idea, we investigated 2 predictions: (1) there should be an equal frequency of breaks and crosslinks, and (2) the protein-associated breaks should be specific for intercalating agents. The frequency determinations were made possible by the method of analysis that we had just worked out using trans-Pt(II) as a model compound. Both predictions were found to hold. The lesion frequencies were equal within experimental error. The effect was demonstrated with diverse intercalating agents (anthracyclines, ellipticine, actinomycin, ethidium and lucanthone) but did not occur with agents that bind to DNA without intercalation or which inhibit macromolecular syntheses without binding to DNA.

The significance of this new type of effect in DNA may not be in its cytotoxic potential. The extent of cell killing was not related to the frequency of this DNA lesion; for a given lesion frequency, adriamycin produced far more killing of L1210 cells than did ellipticine. The protein-associated break may instead be a physiologic response of cells to DNA intercalation or to changes in DNA coiling.

A possible application of the phenomenon is as a test for DNA intercalation in cells treated at pharmacologic dosage. We plan to examine several active anthracycline analogues that have been reported not to intercalate in tests with isolated DNA to see whether in cells they produce an intercalator-like effect.

The measurement of DNA interstrand crosslinks, which may well have greater cytotoxic significance, than do DNA-protein crosslinks, still needs further development. The elucidation of the mechanism of the DNA-protein assay procedure has helped to define the problem of interstrand crosslink quantitation, and it is clear that the effect of DNA-protein links must be completely eliminated for a secure quantitation of the interstrand lesions by means of the alkaline elution technique. This problem is more complex than we had orginally thought, however, and is under intensive study.

The characterization of the interstrand crosslink assay would be facilitated by the availability of a model compound that produced primarily this lesion, without significant DNA-protein linking or strand breakage. None of the previously studied agents fit this requirement. We therefore initiated an investigation of psoralen plus light, a well-characterized means of generating interstrand crosslinking. This study is being conducted in collaboration with the Pediatric Oncology Branch. By means of the alkaline elution technique, we were able to demonstrate crosslinking in cells much more sensitvely than previously reported. The extent of crosslinking increased with the square of the light exposure, as expected for a 2-photon process which has been shown by others to be required for interstrand crosslinking. The crosslinking showed little reversal by proteinase, as expected for crosslinks primarily of the interstrand type.

A new topic of investigation in this area has been an attempt to measure DNA double-strand breaks by means of the filter elution technique. This type of lesion may be of great significance in cell killing, but cannot be measured using an alkaline technique of analysis. The filter elution procedure was therefore studied at a pH below that which causes DNA strand separation. The results have so far been highly encouraging. DNA elution attributable to double-strand breakage has been observed following treatment of cells with X-ray or bleomycin. The ability of the cells to repair this lesion is under study.

The second objective of the studies of intracellular DNA lesions is to relate these lesions to cell killing, particularly to differential toxicity by given agents on different cell types and to differences between agent analogues. We had previously obtained encouraging results in studies of the effect of MeCCNU on 3 human colon tumor lines having different drug sensitivities. In these previous studies only the total crosslinks, consisting both of interstrand and DNA-protein lesions, were measured. We have now initiated a study of the effects of various nitrosoureas on normal human embryonic cells and an SV40 virus-transformed derivative line. The transformed line was found to be more sensitive than the normal line, and the degree of differential toxicity depended on the type of nitrosourea. In the total crosslink assays, differences were seen that correlate with the toxicity differential. Further investigation has been delayed however because of the need to better define the interstrand crosslink assay. Another approach is being conducted in collaboration with the Clinical Pharmacology Section of the Medicine Branch concerning the effects of *cis-*

and trans-Pt(II) in L1210 cells. In this system, the proteinase-alkaline elution assays clearly showed that 2 different types of DNA lesions are formed and repaired with different kinetics. The quantitation and identity of the proteinase-resistant lesions as interstrand crosslinks however is uncertain. We were nevertheless encouraged to find that, while cis- and trans-Pt(II) produced widely different extents of total crosslinking for the same extent of cell killing, the results with proteinase-resistant crosslinking showed a correspondence between DNA lesions on cell kill.

Studies were conducted in our laboratory to identify the proteins linked to DNA by the platinum(II) complexes. It was found that in cells or nuclei treated with high concentrations of Pt(II) complexes many of the nuclear non-histone proteins become bound to the DNA while the histones remain free. One may speculate that this remarkable difference is due to the propensity of Pt to react with sulfhydryl groups which are lacking or inaccessible in the histones. In order to analyse the bound proteins by gel electrophoresis, it was first necessary to dislodge them from the DNA. We found that this could be accomplished by means of thiourea. The release of the bound proteins by thiourea demonstrated that their binding to DNA was in fact through platinum. The question was then raised as to whether thiourea could also reverse DNA interstrand crosslinking, and this was indeed found to be the case. In these studies, isolated density-labeled DNA was reacted with drug and analysed by isopycnic centrifugation. The biological significance of interstrand crosslinking by cis-Pt(II) was tested in a phage λ DNA transfection assay. The isolated phage DNA was reacted with drug and assayed for interstrand crosslinking by analytical ultracentrifugation and for biological activity by the transfection assay. It was found that there were about 5 times as many inactivating hits per DNA molecule than interstrand crosslinks. Interstrand crosslinks therefore were not the major inactivating lesion. The biological activity however could be recovered by treating the DNA with thiourea, and the recovery was commensurate with the reversal of interstrand crosslink. These results point to the existance of another type of lesion, perhaps intra-strand crosslinks, which may be important in inactivating the DNA.

The structure of chromatin was studied using chemical crosslinking agents to determine proximities between different histone molecules. To facilitate these studies, new methods were developed, including 2-dimensional gel electrophoresis and the fluorographic determination of weakly radioactive spots. A new finding was the proximity of histones H1 and H2a. Another notable finding was that the crosslinkability of H2a-H2b depends on the divalent metal environment of the cell nuclei. This may be an indicator of structure modification of chromatin.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06112-06 LMP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Effects of Chemotherapeutic Agents on DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.1	3.5	1.6

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project aims to characterize the formation and repair of DNA damage after treatment of mammalian cells with antitumor agents and to correlate the effects on DNA with cell killing. In addition to standard techniques of DNA analysis, this work entails the development of a new set of techniques based on the DNA alkaline elution principle. During the past year several advances were made of which the following are most notable: (1) A method of quantitation of DNA-protein crosslinks has been devised and applied to trans-Pt(II) complex and X-ray. (2) A new type of drug-effect on DNA, consisting of protein-associated DNA single-strand breaks, was discovered in cells treated with DNA intercalating agents (e.g. adriamycin and ellipticine). (3) Two different classes of DNA crosslinks were observed in cells treated cis- and trans-Pt(II) complexes.

Project Description:

Objectives:

(1) To determine the effects of chemotherapeutic agents on DNA structure and function in cells.

(2) To determine the effects of drugs on DNA replication and repair mechanisms.

(3) To relate the above effects with lethality in various cells types.

Methods Employed:

(1) Cell culture of various rodent and human cell lines--treatment with chemical agents *in vitro*; radioactive labeling of macromolecules.

(2) DNA damage analysis by alkaline elution methods developed in this laboratory.

(3) X-irradiation of cell cultures.

(4) Preparative and analytical ultracentrifugation.

(5) Measurement of DNA single-strand length distributions by alkaline sucrose gradient sedimentation.

Major Findings:

1) Characterization of the Alkaline Elution Measure of DNA-protein Crosslinks, and Application to *Trans*-Platinum(II) Complex.

DNA-protein crosslinks have long been suspected to play a role in the cytotoxic actions of alkylating agents, ionizing radiation and ultraviolet light. Progress has been limited however by methodology which, being based on crude measurements of reductions in DNA extractability, was insensitive and difficult to quantitate. Our previous annual report described the use of the alkaline elution technique to detect both DNA-protein and DNA interstrand crosslinking. Both types of crosslinks reduce the elutability of DNA single-strands fragmented to a controlled size by means of a low dose of X-ray. DNA-protein crosslinks were distinguished on the basis of the extent to which the change in DNA elutability could be reversed by treatment of the lysed cells with proteinase. During the past year, we have succeeded in characterizing the mechanism of the reduced elutability, and we have developed a quantitative theory according to which the crosslink frequencies can be calculated.

Firstly, we found that, under the conditions of alkaline elution, proteins adsorb to the filters, and that this is prevented by the detergent, Sarkosyl. The retention of protein-linked DNA by the filters was reversed by Sarkosyl at the same time that the protein was released. We inferred therefore that DNA-protein crosslinks can retard the elution of long DNA single strands because of the adsorption of the linked protein to the filter.

Secondly, we developed a simple quantitative model on the assumption that DNA strands linked to one or more protein molecules would be retarded on the filters while free DNA strands elute rapidly. The model was tested in L1210 cells treated with trans-Platinum(II)diamminedichloride, which we found to be a potent crosslinker whose effect was almost completely reversed by proteinase. The test assumed that the frequency of DNA-protein crosslinks produced by the drug was proportional to drug concentration; the range of concentrations used (1-hr treatment of L1210 cells) permitted 10-100% survival of colony formation. After removal of drugs, the cells were chilled and exposed to 2-15 krad of X-ray to induce random single-strand breaks, and then analysed by alkaline elution. This range of X-ray doses reduces the DNA strand lengths to the point that they elute rapidly unless held in some way on the filter. Thus 2-component elution curves were obtained from which the fraction of the DNA retarded--i.e. crosslinked--could be determined. The fraction of the DNA crosslinked depended on trans-Pt(II) concentration and on X-ray dose in the manner expected by theory. Using the drug as a model DNA-protein crosslinking agent, the theory was thus validated and the frequency of the crosslinks was estimated. The method was sensitive enough to detect these crosslinks at drug doses that produced little or no reduction of colony-forming ability, suggesting, as was later verified, that the cells must be able to remove these lesions.

A by-product of this work was a quantitative estimate of the frequency of DNA-protein crosslinks produced by X-ray. Such crosslinks had been known to be produced, but it had not previously been possible to quantitate their frequency.

2) Protein-associated DNA Breaks Induced by DNA Intercalating Agents.

Following up on previous reports that agents such as adriamycin, daunorubicin and actinomycin produce DNA single-strand breaks in cells, we tested the effects of these and other intercalating agents by the alkaline elution method. Unexpectedly, no breaks were detected with adriamycin by the direct alkaline elution procedure while analyses by alkaline sedimentation did indicate drug-induced breaks. Upon inclusion of the proteinase digestion step in the alkaline elution assay, however, the expected DNA breaks appeared, and their frequency was consistent with the alkaline sedimentation measurements. This suggested that the drug-induced breaks are in some way hidden by protein-containing material; in particular, DNA-protein crosslinks could cause DNA retention on the filters, as previously explained, and could thereby hide the presence of DNA breaks in the direct alkaline elution assay. These hypothesized drug-induced DNA-protein crosslinks seemed to have a special relationship to the drug-induced single-strand breaks, since these breaks were totally hidden while an equal frequency of breaks produced by X-raying the drug-treated cells were only partially hidden. A possible model was that the drug produced one DNA-protein crosslink on each single-strand segment between breaks, a particularly attractive possibility being that a protein molecule becomes linked at a specific terminus of each drug-induced single-strand break. Each single-strand segment between breaks would then be linked to a protein molecule and would therefore be held on the filter unless the proteinase digestion step was included in the assay. A test of this hypothesis was that the frequencies of drug-induced DNA-protein crosslinks and single-strand breaks should be equal. We were able to estimate the DNA-protein crosslink frequency by means of the method we had developed for trans-Pt(II) as a model compound (paragraph one above). The method for estimating single-strand break frequency had previously been worked out and reported. The results were consistent with hypothesis and indicated the frequencies of the two lesions to be equal within a factor of 2.

Thus we appar to have observed a new type of drug-induced DNA lesion, a protein-associated strand break. This effect appeared to be specific for DNA intercalating agents: it was produced by adriamycin, daunorubicin, ellipticine, actinomycin, ethidium, and lucanthone, all of which are intercalating agents of widely diverse chemical structure; it was not produced by the non-intercalative DNA binders, chromomycin and anthramycin, nor by inhibitors of macromolecular synthesis which do not bind to DNA, viz. ara-C, cordycepin, methotrexate, hydroxyurea and cycloheximide.

The dependence of protein-associated strand breaks on intercalation suggests a relationship to DNA helix unwinding, an effect common to all intercalating agents. Changes in helix winding can be acted upon by nicking-closing enzymes which produce a single-strand break while the enzymes become covalently linked to one of the DNA termini. Another possibility is that the single-strand breaks result from the action of an endonuclease which becomes bound to a strand terminus. In either case, the breaks may result from a physiological process and may not signify a cytotoxic lesion. Supporting this concept was the finding that, for a given frequency of protein-associated breaks, different intercalating agents can produce widely divergent cell kills. Adriamycin, for example, produced much greater killing of L1210 cells for a given frequency protein-associated breaks than did ellipticine. Adriamycin apparently produces some other lesion having much greater cytotoxic consequence.

The 2 drugs also differed strikingly in the kinetics of formation of the protein-associated breaks. Adriamycin generated a linear increase in lesion frequencies with time for several hours, whereas ellipticine caused a rapid rise after which the lesion frequencies remained consistant, but the significance of this difference is unknown.

3) Proteinase-sensitive and Proteinase-resistant DNA Crosslinks.

The anti-tumor activity of DNA-reactive agents usually require that the agents have the capacity to react bifunctionally. The standard alkylating agents almost universally are bifunctional. This is also true for platinum complexes, which have electrophylic reaction mechanisms analogous to alkylating agents. The chloroethylnitrosoureas also appear to react bifunctionally by their ability to chloroethylate nitrogen or sulfur atoms, resulting in a product that is itself a 2-chloroethylamine, or 2-chloroethylsulfide alkylating group.

All of these classes of agents (except myelran) produce both DNA interstrand crosslinks and DNA-protein crosslinks. DNA interstrand crosslinking has been demonstrated in purified DNA, in cells, and in animals by several laboratories using standard techniques. The study of DNA-protein crosslinks has been facilitated by the alkaline elution technique, and we have found this lesion to be produced in cells treated with all tested agents of the above-mentioned classes.

We posed the question of whether the bifunctionality requirement for cell kill could be attributed to either of the above-mentioned types of crosslinks.

We have attempted to develop an alkaline elution technique that would sensitively measure both DNA-protein and interstrand crosslinks. The measurement of DNA-protein crosslinks now seems secure, based on the reversibility by proteinase and the characterization described in paragraph 1. The measurement of interstrand crosslinks based on the proteinase-resistant crosslinking effect however is less secure, both from theoretical and methodologic viewpoints. It seems likely that the proteinase-resistant component includes interstrand crosslinks, but a contribution by residual links of DNA to protein or other non-DNA molecules is not ruled out. Also, the effect of interstrand crosslinks in the presence of residual DNA-protein crosslinks could be complex. The quantitation of these crosslinks is in any case uncertain.

While studies of this problem are still in progress, we have nevertheless studied the behavior of proteinase-sensitive and resistant crosslinks under standard assay conditions. Two lines of evidence strongly supported the idea that this procedure distinguishes at least 2 different classes of crosslinks. First, the extent to which proteinase treatment reversed the crosslinking effect differed widely, depending on the drug. While crosslinking induced by trans-Pt(II) was largely reversed by proteinase, crosslinking by psoralen plus light (a well-established means of generating interstrand crosslinks) was little affected. Intermediate results were obtained with nitrogen mustards, cis-Pt(II) and chloroethylnitrosoureas. Second, the extent of reversal by proteinase depended on time after drug treatment with platinum complexes or chloroethylnitrosoureas; with both types of agents, proteinase reversibility was high immediately after 1-hr treatments of L1210 cells and diminished over a period of hours thereafter. In the case of chloroethylnitrosoureas, this is consistent with the known delay in the formation of interstrand crosslinks in isolated DNA.

Although the relatonship between the crosslink classes and cell killing requires further study, a first indication was obtained from studies of the effects of cis- and trans-Pt(II) in L1210 cells. With both agents, proteinase-sensitive crosslinking peaked soon after 1-hr treatments and then declined, presumably through the action of DNA repair processes. Proteinase-resistant crosslinking increased gradually, and peaked 12 hours after treatment, after which it too declined as if repaired. For a given extent of cell killing (reduced cloning efficiency in soft agar), trans-Pt (II) produced a much greater extent of proteinase-sensitive crosslinking than did cis-Pt(II). The peak proteinase-resistant crosslinking by the 2 agents however was similar for equal cell killing.

The suggested interpretation is that, for the action of platinum complexes in L1210 cells, interstrand crosslinking correlates with cell killing while DNA-protein crosslinking is relatively inocuous. This does not mean however that it is the interstrand crosslinks per se that are killing the cells. It may be that intra-strand crosslinks, which are not measured by the current methods, are the significant lesions and happen to correlate in frequency with interstrand crosslinks. That this may indeed be the case was suggested by the findings of Drs. Filipski and Bonner on phage λ DNA described in their annual report.

The conjecture that DNA-protein crosslinks are relatively inocuous for the case of platinum complexes should not be generalized to other agents, since the types of proteins bound and DNA sites of binding are likely to differ depending on the agent.

4) Relation Between DNA Lesions and Differential Cytotoxicity.

This key area will be increasingly emphasized as mechanistic questions regarding the DNA lesions are answered.

We have found in a pair of human colon tumor cell lines having different sensitivities to MeCCNU that the extent of DNA crosslinking, measured by the direct alkaline elution method, is greater in the more sensitive line. This difference in the effect of MeCCNU on the DNA was observed both in the xenograft tumors in nude mice and in the corresponding cell lines in culture. The difference may reside in the extent of DNA crosslinking, in the extent of crosslink repair, or both.

Currently we are comparing normal human embryo cells with an SV40 virus-transformed line with respect to the effects of various chloroethylnitrosoureas. Both cell lines were tested by cloning assays to determine differential toxicities, and the transformed line was found to be more sensitive than the normal line to all chloroethylnitrosoureas tested: BCNU, CCNU, chlorozotocin and chloroethylnitrosourea. The magnitude of the difference was greater for chlorozotocin and chloroethylnitrosourea than for BCNU or CCNU. The question is whether corresponding differences exist in DNA lesions following drug. Results so far indicate that, 24 hrs after treatment, the transformed cells have greater extents of crosslinking than do normal cells. The differences with chlorozotocin and chloroethylnitrosourea also appear to be larger than with BCNU or CCNU.

Although encouraging, further developmental work is needed to place these results on a solid basis. This is especially true for proteinase-resistant assays which in these experiments have given scattered data.

5) Studies of DNA Replication by pH-step Elution.

We had previously studied the pH transition zone from pH 11.0 to 11.8 over which nascent DNA single-strands become elutable from cells lysed on filters, the strand size eluted increases with pH. In current studies, we find that the strands eluting at pH 11.0 sediment in the 5-9S range, and appear to represent nascent DNA segments near replication forks ("Okazaki fragments"). The strands eluting at pH 11.3 are of the order of 30 kilobases in size while those eluting at pH 11.5 are 60-90 kilobases; these sizes correspond to roughly one replicon. Larger segments elute at higher pH.

Studies with several cell types suggested that this procedure may be useful in studies of drug effects on replication events and for the isolation on relatively large quantities of replication intermediates for biochemical studies.

Two independent lines of evidence indicate that the pH 11.3 material may be synthesized in the region of replicon origins. Following low doses of X-ray (a known inhibitor of replicon initiation), there is a marked reduction in the amount of material eluted at pH 11.3. A second line of evidence comes from FUdR synchronized cells. FUdR inhibits DNA synthesis, and cells will not replicate their DNA until thymidine is supplied. If cells treated with FUdR are pulse labeled, nearly 50% of the incorporated ^{3}H-thymidine is eluted at 11.3, suggesting that this fraction is enriched with replicon origins.

Proposed Course:

1) Develop suitable quantitative assays for interstrand crosslinking. This will require further study of the alkaline elution system to make secure the distinction from DNA-protein crosslinks and to produce a basis for quantitative measurement. Alternative methods will also be explored for making this measurement.

2) Develop neutral elution method for measurement of DNA double-strand breaks in cells.

3) Develop improved assay for DNA alkali-labile sites.

4) Correlate DNA lesions produced by various drugs on diverse cell lines in order to identify the DNA effects that are responsible for cell killing or to develop predictive sensitivity tests.

5) Relate DNA lesions and cell killing by alkylating agents and platinum complexes in various resistant L1210 lines in mice.

6) Initiate similar studies on solid tumors in mice.

Publications:

1. Erickson, L.C., Bradley, M.O., and Kohn, K.W.: Strand breaks in DNA from normal and transformed human cells treated with 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res. 37, 3744-3750, 1977.

2. Erickson, L.C., Osieka, R., and Kohn, K.W.: Differential repair of 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea-induced DNA damage in two human colon tumor cell lines. Cancer Res. 38, 802-808, 1978.

3. Erickson, L.C., Bradley, M.O., and Kohn; K.W.: Differential inhibition of the rejoining of X-ray induced DNA strand breaks in normal and transformed human fibroblasts treated with 1,3-bis(2-chloroethyl)-1-nitrosourea *in vitro*. Cancer Res. 38, 672-677, 1978.

4. Ross, W.E., Ewig, R.A.G., and Kohn, K.W.: Differences between nitrogen mustard and melphalan in the rates of formation and removal of DNA crosslinks. Cancer Res. 38, 1502-1506, 1978.

5. Zwelling, L., Kohn, K., Ross, W., Ewig, R., and Anderson, T.: Kinetics of formation and disappearance of a DNA crosslinking effect in mouse leukemia L1210 cells treated with *cis* and *trans* diamminedichloroplatinum(II). Cancer Res. 38, 1762-1768, 1978.

6. Kohn, K.W., Ross, W.E., Glaubiger, D.: Ellipticine, in Antibiotics Mechanism of Action, Vol. V (Hahn, F. Ed.). Springer-Verlag, in press, 1978.

7. Kohn, K.W., Ross, W.E., Ewig, R.A.G.: A relationship between DNA single strand breaks and DNA-protein crosslinks in intercalator-treated mouse L1210 cells, in Proceedings of the Keystone Conference on DNA Repair, in press, 1978.

8. Bradley, M.O., Erickson, L.C., and Kohn, K.W.: Non-enzymatic DNA strand breaks induced in mammalian cells by fluorescent light. Biochim. Biophys. Acta, in press, 1978.

9. Erickson, L.C., Bradley, M.O., and Kohn, K.W.: Measurement of DNA damage in Chinese hamster cells treated with equi-toxic and equi-mutagenic doses of nitrosoureas. Cancer Res., in press, 1978.

10. Thomas, C. B., Osieka, R., and Kohn, K.W.: DNA crosslinking by *in vivo* treatment of sensitive and resistant human colon carcinoma xenografts in nude mice treated with 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU). Cancer Res., in press, 1978.

11. Kohn, K.W.: DNA as target in cancer chemotherapy: measurement of

macromolecular DNA damage produced in mammalian cells by anti-cancer agents and carcinogens, in Methods in Cancer Research, Vol. 17 (H. Busch and V. DeVita, Eds.), in press, 1978.

12. Ewig, R.A.G., and Kohn, K.W.: DNA-protein crosslinking and DNA interstrand crosslinking by haloethylnitrosoureas in L1210 cells. Cancer Res., in press, 1978.

13. Ross, W.E., Glaubiger, D.L., and Kohn, K.W.: Protein-associated DNA breaks in cells treated with adriamycin or ellipticine. Biochim. Biophys. Acta, in press, 1978.

14. Gantt, R., Parshad, R., Ewig, R.A.G., Sanford, K.K., Jones, G.M., Tarone, R.E., and Kohn, K.W.: Fluorescent light-induced DNA crosslinking and chromatid breaks in mouse cells in culture. Proc. Nat. Acad. Sci. U.S., in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06132-02 LMP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Biological Effects of Chemotherapeutic Agents on Cultured Human and Rodent Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. O. Bradley	Sr. Staff Fellow	LMP	NCI
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COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Molecular Pharmacology

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2.5	1.2	1.3

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SUMMARY OF WORK (200 words or less - underline keywords)

Our work has shown that the nitrosoureas CNU and CHLZ produce much greater differential toxicities in a normal/transformed human cell system than do other closely related analogs such as CCNU, BCNU, MNU, and FCNU. We are currently attempting to discern the molecular basis for such differential toxicity. We have determined that thymidine is highly mutagenic to mammalian cells and have shown that the mechanism is due to nucleotide base imbalance, since the effects are reversed by deoxycytidine. We have shown that mutation by nitrosoureas in mammalian cells is correlated with DNA single strand-breaks but that cytotoxicity is correlated with DNA-crosslinks. We have developed a new and very sensitive assay for DNA double-strand breaks and have begun to study repair and production of double-strand breaks after both X-ray and bleomycin.

Sister chromated exchanges have been shown not to correlate with either mutations or toxicity, but only with DNA single-strand breaks. We have shown that radical scavengers will reduce the production of DNA strand-breaks by sunlight and fluorescent light. Thus, both light sources may produce intracellular radicals which cause DNA strand-breaks.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

One objective is to pursue the differential toxicity produced by certain nitrosoureas in our normal/transformed human cultured cells. We will continue determining the quantitative degree of differential toxicity produced by other agents and then attempt to relate these findings to drug structure and types of DNA damage. These studies should lead to an increased understanding of the basis of differential toxicity.

We are collaborating with Dr. Donald Reed at Oregon State University on one potential mechanism of differential toxicity, i.e., that the amount and/or rate of generation of reduced thiol is greater for the normal cells and that this determines their greater resistance to certain agents like CNU and CHLZ. We will treat the cells with drugs giving varying degrees of cytotoxicity and he will analyze the amount of glutathione and other thiols by HPLC.

We will examine the role of differential DNA damage and repair in the response of the two cell types to the agents giving the greatest differential toxicity, CNU and CHLZ, and to agents giving the smallest differential, i.e. BCNU and CCNU.

A second major objective is to continue developing our assay for low levels of double strand DNA breaks. This assay is much more sensitive than other available methodologies and will permit us to examine various drugs for their ability to produce double strand breaks. Our objective is to see whether there is any relationship between double strand breaks and anti-tumor effect.

A third objective is to continue studies on the mutagenicity of anti-tumor agents and other compounds. Our goal is to understand the types of DNA lesions that lead to mutagenicity and whether these can be differentiated from lesions causing cytotoxicity and anti-tumor effect. We are beginning studies on anti-mutagenesis with the hopes of understanding how to reduce the deleterious mutagenic/carcinogenic effect of various agents.

Methods:

(1) Cloning and monolayer culture of WI-38, VA-13, CHO, and CHL cells.

(2) Mutation assays using the V-79 Chinese Hamster Lung cell and 6-thioguanine resistance.

(3) Measurements of DNA damage and repair using alkaline elution and alkaline sucrose gradients.

(4) Inverted phase photomicroscopy

(5) Incorporation studies of radioactive drugs and DNA, RNA, and protein precursors.

(6) Measurements of DNA double-strand breaks using the newly developed neutral elution technique.

Major Findings:

(1) A superabundance of exogenous thymidine is both toxic and highly mutagenic to V-79 Chinese hamster cells, but hydroxyurea, an inhibitor of ribonucleoside disphosphate reductase, is toxic but not mutagenic. Hydroxyurea is much more toxic than thymidine on an equimolar basis. Deoxycytidine, by itself, is not toxic at concentrations up to 10 mM. However, 3 mM deoxycytidine completely reversed the toxicity of 10 mM thymidine. The number of 6-thioguanine resistant, presumably mutant, colonies begins to increase at concentrations greater than 10^{-3} M thymidine. At higher concentrations the number of mutants induced rises rapidly. At survival values >65% (10^{-5} to 10^{-3} M thymidine) no dose dependent increase occurs in the mutant frequency nor are the values significantly different from the control values. These data suggest that toxicity may be induced at doses of thymidine that do not produce mutations. Hydroxyurea, on the other hand, was not mutagenic at any concentration tested spanning a wide range of toxicities. Deoxycytidine itself is not mutagenic at concentrations of up to 10 mM. However, 3 mM deoxycytidine will greatly suppress the induction of mutants by 10 mM thymidine. This result suggests that thymidine is mutagenic because it reduces the deoxycytidine pool and that resupplying this pool with exogenous deoxycytidine reverses the effect. Our working hypothesis based on these data is that large pool imbalances can cause mutation because of base substitution, that decreased pool sizes during DNA-synthesis can be toxic, and that decreasing DNA synthesis by itself is not necessarily either toxic or mutagenic.

(2) One way to make chemotherapy more effective is to achieve selective killing of malignant but not normal human cells. In order to achieve such differential toxicity it should help to first understand the various mechanisms by which it can occur. Ideally, such differential toxicity would depend upon a common set of properties that distinguish malignant cells from normal cells. If one could understand what properties these were and how they could be exploited by various agents to elicit selective killing, then one could more effectively treat malignant disease.

As one step in this direction, we have developed an assay for determining whether any given compound is differentially toxic to normal and transformed human cell lines. The assay measures the plating efficiency of the two lines after treatment with various agents. In this way we can make quantitative measurements of the exact differential survival between normal and malignant human cell lines in culture.

The results show that 1-(2-chloroethyl)-1-nitrosourea and 2-[3-(2-chloroethyl)-3-nitrosoureido]-D-glucopyranose are highly toxic to the transformed cells but only slightly toxic to the normal ones. The nitrosoureas, CCNU, FCNU, BCNU, and MNU produced much smaller differential toxicities. Whether these results are generalizable to other normal and transformed human cells remains to be determined. However, they provide a system for understanding at least one mechanism by which differential toxicity can be achieved.

(3) Bradley and Sharkey (Nature 1977) 266: 724-726) demonstrated that fluorescent light is mutagenic to V-79 hamster cells. Recently we have shown that flourescent light exposure produces DNA strand breaks in those cells. We now report that in human IMR-90 fibroblasts DNA strand breaks are produced by fluorescent light and sunlight when the cells are exposed to either light source at 0°C. DNA breaks were detected using the alkaline elution technique (Kohn *et al*. (1976) Biochem. 15: 4629-4637). Thirty minutes of sunlight exposure produced DNA strand breaks comparable to 120' of fluorescent light. The intensities were 188 J $sec^{-1} m^{-2}$ for sunlight and 17 J $sec^{-1} m^{-2}$ for fluorescent light using a UV Products Inc. J 221 Black Ray meter. The DNA strand breaks can be mimicked by treating V-79 cell with H_2O_2 at 0°, but when H_2O_2 treatment was done at 37° few DNA breaks were observed. This observation implies that H_2O_2 produces DNA strand breaks at both 0°C and 37°C but that the breaks are rapidly rejoined at 37°C because of an active repair system. If any of the radical scavengers, dimethyl sulfoxide, glycerol, or potassium iodide, are present during fluorescent light exposure, most of the DNA breaks are prevented. These observations suggest that fluorescent light and sunlight may produce intracellular radicals such as hydroxyl radical and perhaps peroxides, which attack DNA and produce strand breaks. Whether or not these types of lesions subsequently lead to toxicity and mutation remains to be determined.

(4) Recent studies have shown that a variety of strong mutagens can produce sister chromatid exchanges (SCEs) in mammalian cells. Many workers have, on the basis of such results, concluded that agents inducing SCEs are necessarily mutagenic. However, we have found a number of agents that damage DNA in different ways but are neither mutagenic nor in some cases, toxic. For instance, in V-79 cells, trans-Pt(II) produces toxicity, DNA-protein crosslinks, DNA single-strand breaks, and small numbers of DNA-interstrand crosslinks, but is not mutagenic. Hydrogen peroxide produces large numbers of DNA single strand breaks at concentrations that are toxic but not mutagenic in V-79 cells. Light of wavelengths greater than 345 nm is neither toxic nor mutagenic to V-79 cells but produces DNA single-strand breaks. However, all of these agents produce varying numbers of SCEs. These results demonstrate, therefore, that SCEs can be produced by DNA damaging agents that are not necessarily either toxic or mutagenic. There is no necessary correlation between SCEs and mutation, so that much previous work will need to be reexamined.

(5) The DNA of V-79 Chinese hamster cells was examined by alkaline elution following treatment of cultures with 8 different nitrosoureas. Drug incubations were performed under consistent biological conditions of equal toxicity and equal mutation induction at the HGPRT locus. All of the compounds tested produced to some degree lesions which were observed as DNA strand breaks upon exposure of the DNA to alkali. DNA cross-linking was observed with the haloethylnitrosoureas, but not with methylnitrosourea or streptozotocin. Since methylnitrosourea and streptozotocin required

approximately 40 times the drug concentration to produce toxicity equal to the haloethylnitrosoureas it appears that the ability to crosslink DNA confers increased cytotoxicity to the haloethylnitrosoureas.

(6) A new assay for double-strand DNA breaks has just been developed. The assay is based on the elution through filters of completely deproteinized DNA at pH 9.75. The sensitivity of the assay is approximately equivalent to 500 Rad of X-ray, and so represents a 25-100 fold increase in sensitivity relative to previous neutral sucrose gradient methods. So far, we have shown that DNA double strand breaks are produced by X-ray, hydrogen peroxide, and bleomycin, that X-ray and bleomycin induced breaks are repaired in rodent cells; and that the eluted DNA is in fact double-stranded in neutral CsCl gradients. This assay opens up a wide variety of problems for experimental attack.

(7) We have coupled DNA-alkaline elution to assays for endonuclease-sensitive sites using purified *M. luteus* endonculease. Thus far, we have shown that the assay can be used to detect breaks produced by the enzyme after cells have been irradiated with germicidal and fluorescent light. Such endonuclease sensitive sites imply that thymine dimers are present, so that the mutagenicity and toxicity of fluorescent light could be due to these potent lesions.

(8) We are developing an assay, whereby various drugs and carcinogens can be reacted with DNA directly on our elution filters. MNU makes 100 times more breaks or alkaline sensitive sites in DNA than it does in identically treated cells. We are attempting to couple the naked DNA assay with an activating system of human monocytes and other tissues. If such a system can be worked out, it should be useful not only as a carcinogen screen but also as a way to ask many questions about metabolic activation of pharmacologically interesting drugs by human tissues.

Proposed Course:

(1) To study the basis for the differential toxicity exhibited by CNU and CHLZ towards transformed human cells. We will look at both DNA damage and repair with the alkaline elution methodology and also at glutathione levels before and after drug treatment.

(2) We will continue to develop the DNA double-strand break assay. Once the assay is well characterized we will use it to study a variety of different agents for their ability to cause double-strand breaks and for the kinetics of double-strand break repair after damage.

(3) The mutagenesis work is winding-down, but during part of this year we will continue to explore mechanisms of mutagenesis by alkylating agents and the possibilities of preventing alkylating mutagenesis with potential anti-mutagens.

Publications:

1. Bradley, M.O., Kohn, K.W., Sharkey, N.A., and Ewig, R.A.: Differential cytotoxicity between transformed and normal human cells with combinations of aminonucleoside and hydroxyurea. Cancer Res. 37, 2126-2139, 1977.

2. Bradley, M.O.: Regulation of protein degradation in normal and transformed human cells: effects of growth state, medium composition and viral transformation. J. Biol. Chem. 252, 5310-5315, 1977.

3. Erickson, L.C., Bradley, M.O., and Kohn, K.W.: Differential inhibition of rejoining of X-ray induced DNA strand breaks in normal and transformed human fibroblasts treated with BCNU *in vitro*. Cancer Res., 38, 672-677, 1977.

4. Erickson, L.C., Bradley, M.O., and Kohn, K.W.: Strand breaks in DNA from normal and transformed human cells treated with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). Cancer Res. 37, 3744-3750, 1977.

5. Bradley, M.O., Erickson, L.C., and Kohn, K.W.: Non-enzymatic DNA strand breaks induced in mammalian cells by fluorescent light. Biochim. Biophys. Acta, in press, 1978.

6. Bradley, M.O., Sharkey, N., Kohn, K.W., and Layard, M.: Cytotoxicity and mutagenicity of various nitrosoureas in V-79 Chinese hamster cells. Cancer Res,, in press, 1978.

7. Erickson, L.C., Bradley, M.O., and Kohn, K.W.: Measurement of DNA damage in Chinese hamster cells treated with equi-toxic and equi-mutagenic doses of nitrosoureas. Cancer Res., in press, 1978.

8. Bradley, M.O.: Mutagenicity of thymidine to cultured Chinese hamster cells. Nature, in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06140-02 LMP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Protein Interactions in Chromosomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. M. Bonner	Sr. Investigator	LMP NCI
	J. Filipski	Visiting Scientist	LMP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Pharmacology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES X (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Protein interactions in chromosomes are being studied with crosslinking reagents and chemotherapeutic agents. A method has been developed for isolating and characterizing proteins crosslinked to DNA. Nitrogen mustard was found to crosslink certain nonhistone proteins, and possibly one histone to DNA. Cis and trans-Pt(II) also cause extensive DNA-protein crosslinking. Cis and trans-Pt have different biological effects on λ DNA as well as on mammalian cells. With trans-Pt(II), inactivation of λ DNA is directly proportional to the number of molecules with interstrand crosslinks. With cis-Pt (II) the major lethal event is not interstrand crosslinks but probably an intrastrand lesion. Thiourea will reverse all Pt(II) induced lethal lesions.

Histone-histone interactions are also being studied with crosslinking reagents. Water soluble carbodiimide crosslinks histones in chromosomes to one another, one major product being a H1-H2a dimer. Other products are being characterized to obtain some insight into histone-histone interactions.

-6040
(Rev. 10-76)

Project Description:

Objectives:

The overall objectives are to help elucidate the role of chromosomal proteins in the structure and function of chromosomes, and to apply this knowledge to understanding and treating cancer. Specific objectives are to:

(1) Determine the effect of chemotherapeutic agents on the DNA-protein interactions.

(2) Determine the spatial relationship of histones and nonhistone proteins in chromosomes.

(3) Determine the mechanism of Pt(II) biological effects.

Methods:

(1) Crosslinking of protein to protein using bifunctional reagents.

(2) Separation of crosslinks on acrylamide gels.

(3) Efficient recovery of proteins from gels (method developed in this laboratory).

(4) Fingerprint analysis to determine the identity of proteins in crosslinked structures.

(5) Efficient detection of H^3 and C^{14} on thin layer plates (method developed in this laboratory).

(6) Crosslinking of protein to DNA using bifunctional chemotherapeutic agents.

(7) Isolation of proteins crosslinked to DNA by centrifuging through CsCl.

(8) Development of phage λ DNA transfection as a model system for the biological effects of chemotherapeutic agents. In this system phage λ DNA enters directly into the bacterium and produces a lytic infection. Therefore the biological effects of chemotherapeutic and other agents on pure DNA can be unequivocally determined.

(9) Determination of interstrand crosslinks in λ DNA by analytical centrifugation.

Major Findings:

1) Proteins Crosslinked to DNA.

While protein-DNA crosslinks can be detected by alkaline elution, the identity of the proteins crosslinked to DNA can not yet be determined by this method. During the previous fiscal year, we developed a method for determining the identity of proteins crosslinked to DNA after treatment of cells with nitrogen mustard (HN2) (Reference 4).

During this fiscal year, we have applied this method to the study of cis and trans-Pt(II) induced DNA-protein crosslinks. Cis and trans-Pt(II) were found to be similar to HN2 in that mainly nonhistones were crosslinked to DNA; however the results were much more clear cut. Under certain conditions, almost all the nonhistones can be crosslinked to DNA while the histones remain uncrosslinked. Trans-Pt(II) was found to be approximately 5 times more efficient than cis in protein-DNA crosslinking.

2) Reversability of Pt(II) Induced Crosslinks.

In order to analyze the proteins crosslinked to DNA after Pt(II) treatment, it is necessary either to destroy the DNA or to reverse the crosslink so that the proteins can be liberated. We tested several compounds known for their ability to complex Pt(II) avidly, and found that thiourea does release proteins bound to DNA after Pt(II) treatment. This new technique enabled us to easily analyze these proteins and also provided evidence that the linkage was in fact through Pt(II).

In further studies thiourea was also found to reverse DNA-DNA crosslinks. However, we wanted to know whether thiourea could remove all lethal effects of Pt(II). To study this aspect, the λ DNA transfection assay was introduced (see Methods). We found that λ DNA which had been treated with cis-Pt(II) to 0.5% activity could be regenerated to full activity with thiourea (1 M, 37°C, 60 hours), although high concentrations of thiourea and long incubation times were necessary. Therefore we conclude that thiourea can reverse all Pt(II) induced lethal lesions in DNA.

One interesting possibility arising from these studies involves the use of thiourea or a similar compound to reverse the lethal effects of Pt(II) on whole cells. If such experiments prove fruitful, thiourea or a similar compound may ultimately be a useful adjunct in Pt(II) chemotherapy.

3) Comparative Effects of Cis and Trans-Pt(II) on DNA.

The λ DNA transfection assay is also being used to compare the lethal effects of cis and trans-Pt(II) on λ DNA. We are confident that these experiments can give some insight into the biological effects of cis and trans-Pt(II) because their relative abilities to inactivate λ DNA are virtually the same as their realtive abilities to kill mammalian cells in culture. In addition, the use of analytical ultracentrifugation enables us to compare the fraction of DNA molecules with interstrand crosslinks with the fraction inactivated. With trans-Pt(II) there is one inactivating event for each interstrand crosslink, indicating that the interstrand crosslink is the major or sole trans-Pt induced lethal lesion in DNA. On the other hand, with cis-Pt(II) there is approximately 5 inactivating events for each interstrand crosslink. This indicates that not interstrand crosslinks but another lesion is the major lethal event. Intrastrand crosslinks are a good possibility for the other cis-Pt(II) induced lethal lesion. In all these cases, thiourea will restore λ DNA to full transfectivity, although the process is much slower with cis-Pt(II) than with trans-Pt(II).

3) Chromosome Structure.

During this past fiscal year, the analysis of histone-histone proximities in chromosomes has greatly advanced. First the development of 2 D gels for the analysis of crosslinked species has allowed the isolation of pure material in many cases for the first time. Secondly, the development of efficient fluorography has allowed the characterization of these structures. One important finding to come from these studies is the proximity of histone H1 to histone H2a; thereby placing H1 at a definite position on the nucleosome core (H2a is part of the nucleosome core). We have also found that with the increased resolution now available, chromosomal conformational changes can be studied. For example, nuclei treated with carbodiimide in EDTA contain an H2a-H2b crosslinked species. However nuclei treated in Ca^{++} do not contain this spot but another one of as yet undetermined structure.

With these new techniques we plan to study differences between euchromatin and heterchromatin, and between normal and neoplastic cells.

Significance to Biomedical Research and Program of the Institute

The DNA protein crosslinks induced by chemotherapeutic agents may be an important aspect of their function. Therefore our understanding of this parameter for these drugs could lead to a fuller understanding of their action and be potentially useful in designing drugs of greater selectivity. To work with cis and Pt(II) on λ DNA may lead to a detailed understanding of the different biological effects of these two isomers. The work on nuclesome structure can lead to a greater understanding of chromosome structure and perhaps indicate differences in normal and neoplastic cells that may be exploited for chemotherapeutic purposes.

Proposed Course:

1. To seek a detailed understanding of the different biological effects of cis and trans-Pt(II) on λ DNA.

2. To study the effect of thiourea on mammalian cells with an ultimate goal to determine if thiourea or similar compound could be a useful adjunct to cis-Pt(II) chemotherapy.

3. To continue the studies on chromosome structure to determine what differences exist in normal and neoplastic cells, and how these differences could be exploited for cancer treatment.

Publications:

1. Bonner, W.M.: Proximity and accessibility studies of histones in nuclei and free nucleosomes. Nucleic Acid Res. 5, 71-85, 1978.

2. Bonner, W.M., and Stedman, J.: Efficient fluorography of H^3 and C^{14} on thin layers. Anal. Biochem., in press, 1978.

3. Bonner, W.M.: Protein migration and accumulation in nuclei. The Cell Nucleus (Ed. H. Busch). Academic Press, in press, 1978.

4. Thomas, C.B., Kohn, K.W., and Bonner, W.M.: Characterization of DNA-protein cross-links formed by treatment of L1210 cells and nuclei with bis-(2-chloroethyl)methylamine (nitrogen mustard). Biochem., in press, 1978.

SUMMARY REPORT OF THE LABORATORY OF TUMOR CELL BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1977 - September 30, 1978

This Laboratory is concerned with six major areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanism involved in neoplastic transformation, including a search for viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information in human leukemic cells; (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell differentiation *in vitro*. (This relates to a major interest of the laboratory: Does the phenotypic abnormality of leukemia in man result from a block in leukocyte maturation?) (6) Based on new information in the literature and from studies within this Laboratory, new approaches to cancer chemotherapy are evaluated in *in vitro* and *in vivo* systems.

During the past year a number of findings were reported by investigators from the Laboratory:

(1) A continuously replicating leukocyte line (HL-60) from the blood of a patient with acute promyelocytic leukemia has maintained its myeloid markers for over a year. This cell line undergoes differentiation with DMSO analogous to the differentiation of Friend erythroleukemia cells with DMSO. This is the first human granulocytic cell line.

(2) Productive infection of HL-60 with primate type-C RNA tumor viruses results in complete loss of myeloid differentiation markers as well as the DMSO effect.

(3) Isolation, partial purification, and characterization of conditioned media from PHA stimulated lymphocytes which promote the growth of T lymphocytes.

(4) Fragments obtained by restriction enzyme digestion of human DNA's (from normal and leukemic tissues) hybridized to RNA from primate type-C RNA tumor viruses (SSV-GALV group).

(5) Similar distribution of FeLV related sequences was observed in both virus negative tumored and normal cats. This system can serve as a model system where virus detection is rare, e.g., man.

(6) A technique for the enrichment of type-C viral sequences of high G+C content by banding of DNA in sodium iodide gradients has been used to enrich high G+C containing proviral sequences in human leukemic tissues for molecular hybridization studies.

(7) A technique of competitive RNA hybridization has been utilized for the detection of viral related RNA in leukemic tissues. The presence of mammalian type-C virus related RNA in tissues of some leukemic patients has been observed.

(8) The technique of in situ hybridization of viral cDNA to RNA of fixed cells has been developed and used for the detection of type-C RNA tumor virus RNA in cells producing virus, and of viral related RNA in human leukemic cells.

(9) Antibodies have been isolated from the surface of human leukemic leukocytes that possess high neutralizing activity against feline and murine leukemia virus reverse transcriptases in cases of CML in blast crisis and often against the enzyme from primate viruses in the case of AML. Antibody activity was removed by goat antihuman IgG.

(10) An antibody prepared against DNA polymerase γ has been useful in distinguishing viral reverse transcriptase and cellular DNA polymerase γ. This antibody shows specific binding to DNA polymerase γ by double antibody binding assay.

(11) Demonstrated the presence of terminal deoxynucleotidyl transferase in CML patients in acute blast phase of the disease, its loss during treatment, and reappearance just before blast crisis. This enzyme is a potential biological marker for following these patients during treatment and remission. Leukemic patients positive for terminal transferase showed response to vincristine-prednisone therapy.

(12) Showed by cell separation studies that terminal deoxynucleotidyl transferase is present in a subpopulation of cells, in normal bone marrow and in leukocytes from patients with ALL.

(13) Demonstrated the association of reverse transcriptase with plasma membrane of virus transformed cells as well as from a patient with acute lymphocytic leukemia. The leukemic enzyme was inhibited by the antibody isolated from these cells.

(14) Antisera has been obtained against the glycoprotein gp70 and p30 of simian sarcoma virus. This antisera is being used to develop a radioimmunoassay for the detection of similar glycoproteins from leukemic cells by competition radioimmunoassays.

(15) Variation in the histone patterns have been observed between normal lymphoid and type-C RNA tumor virus transformed cells. Similar studies are in progress with other cell lines including HL-60, and fresh human leukemic cells.

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06117-06 LTCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert C. Gallo	Chief, Lab. of Tumor Cell Biology	LTCB	NCI
Others:	Robert E. Gallagher	Senior Surgeon	LTCB	NCI
	Prem S. Sarin	Chemist	LTCB	NCI
	David Gillespie	Microbiologist	LTCB	NCI
	W. Carl Saxinger	Microbiologist	LTCB	NCI
	David Strayer	Clinical Associate	LTCB	NCI
	Flossie Wong-Staal	Cancer Expert	LTCB	NCI
	Marjorie Guroff	Cancer Expert	LTCB	NCI
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	Bert Vogelstein	Research Associate	LTCB	NCI
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COOPERATING UNITS (if any) Richard Adamson, Laboratory of Chemical Pharmacology, National Cancer Institute; Stu Aaronson, Viral Carcinogenesis Branch, National Cancer Institute; Robert Ting, Biotech Laboratories; Rolf Neth, University of Hamburg; Natalie Teich, Robin Weiss, Imperial Cancer Research Fund, London, England;

LAB/BRANCH

Laboratory of Tumor Cell Biology

SECTION Section on Cellular Control Mechanisms and Section on Nucleic Acid Hybridization

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
37	21	16

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This Laboratory is concerned with six areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search for viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information to human leukemic cells; (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell cycle characteristics; (5) biochemical and physiological studies on leukocyte maturation in vitro. (This relates to a major interest of the laboratory; Does leukemia in man result from a block in leukocyte maturation?) (6) Based on new information in the literature and from studies within this laboratory, new approaches to cancer chemotherapy are evaluated in in vitro and in vivo systems. This is the ultimate goal of the Laboratory.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OR PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Paul Jacquemin	Cancer Expert	LTCB	NCI
Asok Ganguly	Visiting Fellow	LTCB	NCI
Anna Ruspa Ridgeway	Visiting Fellow	LTCB	NCI
Ricardo Della Fevera	Visiting Fellow	LTCB	NCI
Frank Ruscetti	Cancer Expert	LTCB	NCI
Bernard J. Poiesz	Clinical Associate	LTCB	NCI
Jim Mier	Clinical Associate	LTCB	NCI
Sandra Foote	Staff Fellow	LTCB	NCI

COOPERATING UNITS

Wolfe Prensky, Sloan-Kettering Institute; Dani Bolognesi, Duke University; Ken McCredie, M. D. Anderson Hospital and Tumor Institute; Richard Metzgar, Duke University Medical Center, Maxine Singer, NCI; Bill Hardy, Sloan Kettering, New York; Dick Knight, Univ. College, London; George Van der Woude, NCI; Peter Wiernik, Baltimore Cancer Center, George Bekasi, Mt. Sinai Medical Center, New York; Gianmarco Corneo, University of Milan; Yohei Ito, University of Kyoto; Umberto Torelli, University of Modena; Max Essex, Harvard University; Bill Haseltine, Harvard University, Joel Greenberger, Harvard University.

Project Description:

Objectives

1. It is anticipated that a greater understanding of the processes involved in the molecular control of cellular growth, differentiation, and carcinogenic transformation, including the pathogenesis of human neoplasias, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

3. To develop new concepts of chemotherapy and apply them to animal model systems as rapidly as possible as new information is derived from basic experimental studies.

These objectives have primarily been pursued by the following approaches:

1. Purification and properties of enzymes of nucleic acid metabolism especially DNA polymerases (DNA-dependent and RNA-dependent) of human normal and leukemic cells and of RNA oncogenic viruses to determine ultimately:

    a. The function, characteristics, and number of DNA polymerases in these cells and viruses.

    b. If RNA-dependent DNA polymerase is present in human leukemic cells and, if so, is it the same as the virion enzyme? Can it copy viral 70S RNA? Can it copy cellular messenger RNA? Does it function in gene amplification during cellular differentiation? Is its activity altered in leukemogenesis? If found, is its activity necessary for maintenance of the neoplastic cell? Can it be used as a marker of neoplastic cells? If it is not necessary for maintenance of the neoplastic state will its continued inhibition prevent some relapses of animals or patients in remission, i.e., does the inciting agent in human leukemia (? virus) remain and produce subsequent transformation?

    c. Is the viral enzyme necessary for viral replication and for initiation of transformation?

Several of the above questions have now been answered.

2. Biochemical studies on the properties of the RNA of type-C viruses and on the overall pathway of replication of these viruses. Purposes:

    a. To obtain more information on the mechanism of transcription of this RNA to DNA via reverse transcriptase.

b. To determine if diagnostic probes can be obtained, i.e., is their structure specific enough that we can use this information to find viral RNA in cells?

c. In understanding the mechanisms involved in integration and expression of viral genes, we can plan approaches to interfere with this expression and then evaluate the overall biological effect of this interference. We particularly wish to know if viral expression is required to maintain the cell in the neoplastic state.

3. Pursuing studies leading to an understanding of the origin of tumor viruses, how they acquire their oncogenic potential, how they interact with cells, and how they are transmitted throughout nature. These studies are primarily carried out with techniques of molecular hybridization.

4. Leukocyte differentiation in vitro. The soft agar technique for investigating maturation and proliferation of normal and leukemic human bone marrow cells was recently set up in our laboratory. Attempts are made to study exogenous and endogenous (released from feeder layers of normal cells) factors which affect these processes. Attempts have been made here and in other laboratories to differentiate human leukemic blast cells with apparent success. The implications of this to understanding leukemogenesis and for potential therapeutic approaches are obvious. The mechanisms involved in the maturation process are under study.

5. Growth of leukemic myeloblasts in liquid suspension under the sitmulus of a conditioned media factor produced by human embryonic culture cells.

6. Markers: (a) an immuno-chemical technique for finding reverse transcriptase and other viral macromolecules in intact cells is now being developed. (b) Techniques for detecting viral specific nucleic acids in intact cells are also being developed.

7. Cell separation studies are being carried out to enrich subpopulation of leukemic cells which may contain the type-C RNA tumor virus related markers and other biological markers.

8. Antibodies associated with the membranes of human leukemic cells have been isolated. They are under study as to which protein antigens they interact with, i.e., are they leukemia cell specific, e.g., viral, etc.

Methods Employed

1. Human leukocytes were isolated and purified as previously described (J. Clin. Invest. 48: 105-116, 1969; Science 165: 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Biological Effect of Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334; Blood 37: 282-292, 1971).

2. DNA polymerase activities were purified and characteized as reported (Natue New Biology 240: 67-72, 1972; Proc. Natl. Acad. Sci. 69: 2879-2884, 1972; Proc. Natl. Acad. Sci. 69: 3228-323 1972; DNA Synthesis in vitro, Proceedings of the Second Annual Steenbock Symposium, 1972.

3. Viral reverse transcriptase was purified and studied as described (Nature 234: 194-198, 1971; J. Virol. 12: 431-439, 1973; Biochim. Biophys. Acta 454: 212-221, 1976).

4. Macromolecular synthesis, viability, mitosis in leukemic and normal cells and the effects of specific agents were evaluated as described before (J. Natl. Cancer Inst. 46: 789-795, 197; Science 165: 400-402, 1969).

5. In vitro leukopoiesis is studied by the soft agar technique developed by Paran and Sachs. In addition human myelogenous leukemic leukocytes are propagated in liquid suspension culture, as recently reported (Science 187: 350, 1975).

6. Induction of type-C virus from "non-producer" cells by iododeoxyuridine is carried out essentially as originally described by Rowe and colleagues. Infectious units focus formation and plaque assays for virus are carried out by conventional techniques.

7. Molecular hybridization studies are carried out by conventional and by newly evolved techniques. These include: (a) filter technique with DNA; (b) filter technique with RNA covalently attached (Proc. Natl. Acad. Sci. 70: 3219-3224, 1973); (c) Cesium sulfate gradient analyses; (d) S_1 nuclease treatment; (d) RNA-DNA hybridization by competition analyses (Methods in Cancer Research, Vol. XI).

8. Tissue culture, virus production, cell viability estimates, cloning of cells are all carried out by standard techniques. Established procedures for titering infectious, leukemic viruses (SX test) and transforming sarcoma viruses (focus formation) are routinely performed. Also, virus neutralization procedures are performed by standard procedures.

9. Virus quantitation, virus specific molecules, metabolism of viral RNA and proteins are studied by conventional techniques.

10. Cell separation studies are carried out using ficoll-hypaque gradients, sucrose density gradients and free flow electrophoresis (Lancet 1: 508-509, 1976).

11. Isolation and characterization of chromatin and histone and non-histone proteins is carried out by published procedures (J. Mol. Biol., 27: 431, 1967, and J. Mol. Biol., 67: 361, 1972).

Major Findings

1. A continuously replicating leukocyte line (HL-60) from the blood of a patient with acute promyelocytic leukemia has maintained its myeloid markers for over a year. This cell line undergoes differentiation with DMSO analogous to the differentiation of Friend erythroleukemia cells with DMSO. This is the first human granulocytic cell line.

2. Productive infection of HL-60 with primate type-C RNA tumor viruses results in complete loss of myeloid differentiation markers as well as the DMSO effect.

3. Isolation, partial purification, and characterization of conditioned media from PHA stimulated lymphocytes which promote the growth of T lymphocytes.

4. Fragments obtained by restriction enzyme digestion of human DNA's (from normal and leukemic tissues) hybridized to RNA from primate type-C RNA tumor viruses (SSV-GALV group).

5. Similar distribution of FeLV related sequences was observed in both virus negative tumored and normal cats. This sytem can serve as a model system where virus detection is rare, e.g., man.

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14. Antisera has been obtained against the glycoprotein gp70 and p30 of simian sarcoma virus. This antisera is being used to develop a radioimmunoassay for the detection of similar glycoproteins from leukemic cells by competition radioimmunoassays.

15. Variation in the histone patterns have been observed between normal lymphoid and type-C RNA tumor virus transformed cells. Similar studies are in progress with other cell lines including HL-60, and fresh human leukemic cells.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, these studies are designed to obtain fundamental information on molecular and physiological control mechanism and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

Proposed Course

As described above, some projects will terminate and others will continue to be actively pursued.

Publications

1. Sarin, P. S., Aulakh, G. S., and Gallo, R. C.: RNA tumor viruses and leukemia in man. Excerpta Medica 415: 200-203, 1978.

2. Ruscetti, F. W., Morgan, D. A., and Gallo, R. C.: Functional and morphologic characterization of human T cells continuously grown *in vitro*. J. of Immun. 119: 131-138, 1977.

3. Collins, S. J., Gallo, R. C., Gallagher, R. E.: Continuous growth and differentiation of human myeloid leukemic cells in suspension culture. Nature, 270: 347-349, 1977.

4. Guroff, M., Schrecker, A. W., Brinkman, B. J., Gallo, R. C.: DNA Polymerase γ of human lymphoblasts. Biochem. 16: 2866-2873, 1977.

5. Gallo, R. C.: Viruses and pathogenesis of human leukemia. Schweiz. med. Wschr. 107: 1436-1440, 1977.

6. Gallo, R. C. and Meyskens, F. L: Evidence for the presence of RNA Tumor Virus Components in Human Leukemia. In Ito, Y. (Ed.): Progress in Experimental Tumor Research. Switzerland, Karger Press, 1978, pp. 216-249.

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ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY

DIVISION OF CANCER TREATMENT

October 1, 1977 - September 30, 1978

Seven Branches comprise the intramural Clinical Oncology Program: Clinical Pharmacology, Medicine, NCI-VA Medical Oncology, Pediatric Oncology, Radiation Oncology, Surgery, and the newly-formed Biometric Research Branch. The mission of this program is to conduct clinical and related laboratory research in cancer treatment, including Phase I, II, and III clinical trials, clinical pharmacology of new drugs, combined modality treatment approaches, immunotherapy, and basic studies of tumor cell biology. Integrated into the program are fellowship training opportunities in the clinical subspecialties of Medical, Pediatric, Radiation and Surgical Oncology. The resources of the NIH Clinical Center and a special ambulatory care program for outpatients are available for clinical research. Laboratory research is conducted within the laboratories of the Clinical Center, with the assistance of several support contracts. The remainder of this report summarizes the highlights of clinical and laboratory research over the past year. Please refer to individual Branch annual reports for details.

Program Accomplishments

Office of the Associate Director for Clinical Oncology/Deputy Clinical Director

This office is responsible for program planning, coordination, and administration of the intramural Branches. In addition, all clinical activities of the NCI, including patient recruitment, protocol review, patient care, bed allocation, and the postgraduate oncology training programs are the responsibility of the Deputy Clinical Director.

Several specific projects are also developed by the Associate Director, either collaboratively or independently.

1) Study of Bilharzial Bladder Cancer in Egypt
Six sequential phase II studies in advanced bladder cancer in collaboration with the Cairo Cancer Institute (Dr. Nazli Gad-El-Mawla) have been completed with support from PL-480 funds. Hexamethylmelamine and trenimon are active compounds; methotrexate, bleomycin, adriamycin, VP-16, and cis-platinum are relatively inactive (reported at American Society of Clinical Oncology, April, 1978, and in Cancer Treatment Reports, in press). A continued search for active phase II agents is planned (cyclophosphamide, 5-FU, neocarzinostatin, pentamethylmelamine). In addition, the use of misonidazole as a radiosensitizer is in clinical trial in Egypt.

2) Continued Studies in Burkitt's Lymphoma
A 10-year followup of 103 Ugandan patients has just been completed, confirming the overall long term survival of 60% projected from earlier trials. Also reported this year are the results of 54 American patients with Burkitt's lymphoma (New England J. Med. 297: 75-80, 1979) showing a survival rate similar to the African experience.

3) A 10-year Followup of 45 Ugandan Children with Hodgkin's Disease Treated only with MOPP Chemotherapy
An overall complete response rate of 88% and long term survival of 75% for early stage and 60% for late stage patients were observed. Results in early stage patients are exactly comparable to those achieved at Stanford University with radiotherapy alone (American Society of Hematology, Plenary Session, San Diego, December, 1977 and Cancer, in press). A collaborative study of gonadal dysfunction in boys with Hodgkin's disease treated with MOPP was reported this year (New England J. Med. July, 1978). This study demonstrated both germ cell depletion and Leydig cell dysfunction in pubescent boys, the latter manifested clinically by gynecomastia.

Biostatistics and Data Management Section

Because of increased responsibilities, this section has recently achieved Branch status (Biometric Research Branch) and will expand in personnel. Major accomplishments this year include:

1) Collaborative development of 13 clinical trials

2) Collaborative analysis of 29 clinical studies

3) Continued work on the "Norton-Simon" hypothesis including the development of animal models to test treatment strategies, and use of the kinetic model to predict drug activity in experimental drug screening.

4) Creation of a general data base system for Clinical Center patients entering clinical trials, as well as specific data systems for breast cancer, non-Hodgkin's lymphomas, Hodgkin's disease, and head and neck cancer.

5) Development of improved statistical methods for examining: a) risk of second tumors; b) determination of optimal sample size; c) interpretation of carcinogenesis experiments; d) analysis of cross-over designs; e) phase II clinical trial designs; and f) heterogeneity of patients in clinical trials.

Clinical Branches - Research Highlights

Clinical Pharmacology Branch

1) Intraperitoneal chemotherapy with methotrexate and 5-FU results in a marked peritoneal plasma concentration gradient indicating potential clinical utility in malignancies with intraperitoneal spread (e.g. ovarian cancer).

2) Further study of the bronchiolar Clara cell reveals two additional compounds (3-methyl furan and carbon tetrachloride) are metabolized to highly toxic intermediates. This discovery has important implications both in carcinogenesis (furan is an environmental contaminant) and in chemotherapeutic selectivity.

3) Melatonin prevents DMBA-induced mammary tumors in the rat, and pinealectomy enhances carcinogenesis in this model. A specific melatonin receptor has been identified in gonadal tissue. These discoveries have important implications in the pathogenesis of mammary cancer in man.

4) Following the discovery that vitamin E prevents adriamycin-induced cardiac toxicity, a search for a clinical application was begun. N-acetyl cysteine is a similar "free radical scavenger" and will be tested in clinical trial this year.

5) Methotrexate pharmacokinetics at the cellular level reveal reversible binding to reductase in intact cells as opposed to slow reversal using purified enzyme.

Medicine Branch

1) The use of the HEXA-CAF drug combination in ovarian cancer more than doubles the complete response rate and median survival (currently 29 months) compared to single agent therapy.

2) The absence of estrogen receptors (ER) in patients with metastatic breast cancer is a powerful determinant of a favorable response to chemotherapy, independent of other prognostic factors. Thus, ER status must be considered in stratification employed in prospective clinical trials. Further, responses to endocrine therapy in ER positive patients are directly related to quantity of receptor present.

3) Combination chemotherapy (CVP) is equivalent to total body irradiation plus CVP in patients with nodular lymphoma.

4) Anti-thymocyte globulin may be effective therapy in T cell lymphomas.

5) Long-term followup of Hodgkin's disease reveals a direct relationship between first remission duration and survival. Similar followup in non-Hodgkin's lymphoma reveals an appreciable incidence (25%) of histologic conversion to less favorable subtypes. A new treatment strategy is now being studied to take advantage of this phenomenon.

NCI-VA Medical Oncology Branch

1) Treatment of small cell bronchogenic cancer with alternating drug combinations (CMC-VAP) yielded a 28 month survival of 23% for patients who achieved a complete response. Survival was prolonged by the addition of thymosin, 60 mg/M^2, during induction. Improved treatment strategy is based on more comprehensive knowledge of the natural history of the disease, including cell doubling time, effect of histologic subtypes, organ involvement, and infectious complications.

2) High dose methotrexate was administered to 28 patients with non-small cell bronchogenic cancer with only 2 partial responses observed.

3) Treatment of gastric cancer with 5-FU and methyl CCNU alternating with adriamycin and mitomycin C yielded an 11% response rate in 18 patients.

4) Treatment of prostate cancer with adriamycin and cyclophosphamide yielded a 35% objective response rate in 20 patients.

5) Combination chemotherapy plus electron beam therapy has been administered to 22 patients with mycosis fungoides or Sezary syndrome with dramatic improvement.

Pediatric Oncology Branch

1) The results of a protocol for the treatment of acute lymphocytic leukemia, initiated in 1971, indicate that 80% of children with "good prognosis" factors are in a durable remission and probably cured. The majority of these patients have not been treated with chemotherapy for the past 4 years. Significant neurotoxicity following CNS prophylaxis mandates a continued search for effective but less toxic approaches.

2) Extraordinary high dose chemotherapy may be effective where conventional dose therapy has failed: Five of 23 patients with relapsed Burkitt's lymphoma have experienced durable remissions after such a therapeutic approach. Mortality is virtually 100% in this clinical setting with conventional treatment.

Human bone marrow can be cryopreserved, stored for long periods, reinfused into the autologous host, engrafted, and can hasten hematopoietic recovery after chemotherapy-induced marrow hypoplasia. These results have now been confirmed in patients with Burkitt's lymphoma and Ewing's sarcoma and permit the safe administration of high dose chemotherapy.

3) HLA matching is not always reliable in predicting compatibility between platelet donors and recipients, since there appear to be other non-HLA antigens that determine the outcome. A new assay (platelet migration inhibition) has been shown to be 100% reliable in predicting compatible transfusions; the assay detects all platelet antigens.

4) Using sophisticated hybridization techniques, the human alpha-globin structural gene has been assigned to chromosome 16, and the human beta-globin structural gene to chromosome 11. These results represent landmark experiments in molecular biology as it applies to human biology and disease.

5) A new strain of Epstein-Barr virus, AG876, was isolated from a fresh Burkitt tumor from Ghana. The new strain transforms cells to malignancy, but is not genetically deleted with respect to nontransforming strains. It is probable that AG876 is the true wild-type prototype of oncogenic EBV. All other strains studied in laboratories throughout the world have probably undergone mutation, and certain of their biological properties may therefore be spurious.

Radiation Oncology Branch

In September 1, 1977, Dr. Eli Glatstein became the Chief of this Branch following the departure of Dr. Johnson. New plans for the Branch include: 1) major emphasis on collaborative clinical trials of combined modality therapy; 2) development of clinical trials in radiation therapy utilizing hyperfractions, radiosensitizers, and hyperthermia; 3) development of a radiobiology laboratory; and 4) creation of a radiation therapy fellowship. Accomplishments during the past transitional year are highlighted below (collaborative studies are reported under other Branch accomplishments).

1) Development of the necessary technology to begin computer assisted treatment planning using a whole-body EMI scanner

2) The achievement of a 35% survival for patients with small cell bronchogenic cancer of limited extent using radiation therapy and combination chemotherapy

3) Total body irradiation plus chemotherapy is highly effective in the management of patients in the accelerated phase of chronic lymphocytic leukemia

Surgery Branch

1) A clinical trial of adjuvant high-dose methotrexate in 39 patients with osteosarcoma reveals a doubling of disease-free interval (44%) as compared to historical controls (18%) at 2 years. Nearly two-thirds of these patients are surviving with no evidence of disease at two years.

2) A clinical trial of 55 patients with resectable soft-tissue sarcoma has shown that disease-free survival is equivalent for patients undergoing radical surgery plus adjuvant chemotherapy as compared to limb-sparing surgery followed by post-operative radiation and chemotherapy. Three-year survival (actuarial analysis) is greater than 90% for this group of patients; this result contrasts to a 50% for patients treated by surgery alone in the past.

3) In a trial of 24 patients with advanced testicular cancer, preliminary evidence points to a beneficial role of debulking surgery prior to combination chemotherapy.

4) A osteosarcoma-specific antibody-antigen system has been discovered which will have important implications for immunotherapy and immunodiagnosis.

5) The first prospective randomized study of total parenteral nutrition (TPN) now includes over 30 patients; and results in dramatic nutritional improvement. The impact of TPN on the clinical outcome is yet to be evaluated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06308-07 CO

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

BIOSTATISTICS AND DATA MANAGEMENT SECTION

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Simon	Head, Biostatistics and Data Management Section	COP, DCT, NCI
OTHER:	Y.J. Lee	IPA Consultant	COP, DCT, NCI
	R.W. Makuch	Staff Fellow	COP, DCT, NCI

COOPERATING UNITS (if any) Clinical Branches, COP, DCT, NCI: Developmental Therapeutics Program, DCT, NCI; Cancer Therapy Evaluation Program, DCT, NCI; Baltimore Cancer Research Center, DCT, NCI; Immunology Branch, DCBD, NCI

LAB/BRANCH

SECTION

Biostatistics and Data Management Section

INSTITUTE AND LOCATION

NCI, National Institutes of Health, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of the section is to utilize statistical and mathematical methods for the development and evaluation of effective treatments for cancer. As part of this research effort, the section designs and analyzes therapeutic trials, conducts studies of important factors in the natural history of these diseases, designs and maintains computerized data collection systems, and develops new biometric methods. A list of current projects follows.

PHS-6040
(Rev. 10-76)

1. Design of Clinical Trials

1.1 The section collaborates in the design of intramural clinical trials. The following studies have been developed during this year: (1) Double blind evaluation of transdermal scopolamine as an anti-emetic; (2) Comparative evaluation of serial CEA, EMI scan and physical examination for detecting recurrences of colorectal cancer. Evaluation of radiotherapy adjuvant for such patients with completely resectable recurrences; (3) Comparative evaluation of two combination chemotherapy regimens for inducing complete remissions in patients with extra-pelvic ovarian carcinoma, and evaluation of large volume intraperitoneal methotrexate for prolonging remissions and eliminating minimal residual disease; (4) Phase II evaluation of DTIC + actinomycin D for neuroblastoma; (5) Evaluation of delayed chemotherapy in the treatment of patients with NPDL lymphoma; (6) Randomized evaluation of the usefulness of HLA matching in the platelet transfusion support of thrombocytopenic cancer patients; (6) Randomized evaluation of sulfa for the treatment of pulmonary disease in patients with metabolic disorders; (7) Randomized evaluation of orchiectomy with hormonal therapy and chemotherapy compared to orchiectomy alone in patients with cancer of the prostate; (8) Randomized evaluation of radiotherapy with or without thymosin for the treatment of patients with carcinoma of the nasopharynx; (9) Randomized evaluation of radiotherapy with or without a radiosensitizer (misonidazole) for patients with squamous cell carcinoma of the esophagus confined to the mediastinum; (10) Randomized evaluation of re-induction pulsing versus conventional continuous maintenance for prolonging remissions of patients with acute lymphoblastic leukemia; (11) Randomized evaluation of prophylactic and oral hygiene for the reduction of oral mucocytis subsequent to chemotherapy in cancer patients; (12) Evaluation of prednisone on pituitary function in patients with acute lymphoblastic leukemia; (13) Randomized evaluation of the effect of disease education and supportive psychotherapy for primary breast cancer patients on their adjustment to mastectomy.

In addition to these collaborations, the section has a representative serve on the clinical research committee to review all intramural clinical trials.

1.2 The section reviews selected extramural clinical trials submitted for approval to the Cancer Therapy Evaluation Program by cooperative groups and DCT contractors.

1.3 The section participates in the development of major national clinical trials. During the past year, this has included the planning of inter-group studies of the treatment of testicular cancer, and a national study of the treatment of stage II-III adenocarcinoma of the head or neck.

2. Statistical Analysis

2.1 Clinical Studies:

The section consults and collaborates in the analysis of clinical and laboratory studies. Collaborations this year have included the following areas: (1) Evaluation of the role of estradiol, progesterone, glucocorticoid and androgen receptors on tumor cells in the natural history, tumor biology and response to therapy of breast cancer patients; (2) Retrospective analysis of all non-Hodgkin's lymphoma patients ever seen at the clinical center. Comparison of 5 pathologic classification systems. Evaluation of the meaning of change in patient histology in the natural history of the disease. Correlation of pathologic classification to degree of dissemination and other patient characteristics. Determination of paths of disease spread, etc; (3) Evaluation of the effect of tumor grade and degree of differentiation on the natural history of patients with stages III-IV ovarian cancer. Evaluation of the usefulness of tumor grade for selecting therapy; (4) Comparison of limb preserving surgery with radiotherapy versus amputation for patients with soft tissue sarcomas of extremities. Evaluation of adjuvant chemotherapy after local therapy in soft tissue sarcoma patients; (5) Evaluation of radiotherapy with or without chemotherapy for patients with non-resectable limited extent bronchogenic carcinoma (all four major histologies evaluated separately); (6) Evaluation of delta-9 tetrahydrocannabinol as an anti-emetic in patients with osteogenic sarcoma receiving high dose methotrexate; (7) Evaluation of thymosin as an adjuvant to the use of combination chemotherapy in the treatment of oat cell bronchogenic carcinoma. The effect of thymosin on tumor response, survival, wbc counts, T-cell levels and α_2-HS levels were evaluated; (8) Evaluation of laminar air flow isolation for reducing infectious complications and prolonging survivals of cancer patients receiving intensive chemotherapy; (9) Evaluation of the effect of duration of administration of broad spectrum antibiotics for febrile granulocytopenic leukemic children without documented infection on clearance of febrile episode and development of super-infection. Evaluation of causes of fevers of unknown origin and diagnostic procedures of value in this population; (10) Evaluation of HLA antigens in the etiology and natural history of acute non-lymphoblastic leukemia; (11) Serial evaluation of the effect of BCG on the immune response of patients with resectable osteogenic sarcoma. Evaluation of immunological surveillance programs for the early detection of tumor dissemination in such patients; (12) Multivariate evaluation of stage, age, sex, lactate, LDH, uric acid, anti early-antigen titre and anti VCA titre on the prognosis of patients with Burkitt's lymphoma; (13) Interim analysis of prospective randomized clinical evaluation of chemotherapy or immunotherapies as adjuvant treatments for patients with stage II melanoma; (14) Multivariate analysis of tumor and host characteristics and treatment intensity on the prognosis of patients with Ewing's sarcoma; (15) Serial evaluation of the effect of whole body hyperthermia on lymphocyte and T-cell counts; (16) Evaluation of the effect of whole body hyperthermia, BCNU chemotherapy and both combined on the _in vitro_ colony forming capacity of human bone marrow; (17) Serial evaluation of changes in serum zinc and copper levels in 19 tumor bearing patients receiving total parenteral nutrition; (18) Retrospective evaluation of the efficacy of pre-operative methotrexate in 60 patients with "resectable" head-neck cancer; (19) Evaluation of serum levels of α_2-HS glycoprotein and seven acute phase proteins as potential correlates of tumor growth and immune reactivity in 150 cancer patients; (20) Comparison of cytofluorgraphic analysis and light microscopy counting for quantitation of T-cell and B-cell serum pools; (21) Analysis of a Phase I-II study of cis-platinum in 40 patients with ovarian and breast carcinoma; (22) Retrospective evaluation of thoracotomy for the diagnosis and treatment of lung lesions in melanoma patients; (23) Serial evaluation of the effect of colchicine on granulocyte levels in cancer patients; (24) Evaluation of serial CEA evaluations for predicting prognosis in 31 patients with oat cell carcinoma of the lung receiving radiotherapy; (25) Evaluation of the diagnostic utility and complications of peritoneoscopy for the staging of cancer patients; (26) Multivariate evaluation of pre-therapy CEA, hemoglobin, albumin, α_1-protein and LDH as prognostic factors from 65 patients with oat cell carcinoma of the lung; (27) Multi-institution retrospective analysis of prognostic factors in the treatment of advanced colo-rectal carcinoma; (28) Evaluation of the effect of radiotherapy to sarcoma patients on the Pha response of cyropreserved lymphocytes; (29) Evaluation of high dose methotrexate as a surgical adjuvant in the treatment of patients with osteosarcoma.

2.2 Animal Studies:

(1) Design and analysis of a study to evaluate the effect of diet on gastrointestinal transit time and carcinogenic effect of 1-2 dimethylhydrazine in rats. (2) Design and analysis of a study to evaluate the protective effect of BCG on granulocytes of mice receiving chemotherapy. (3) Evaluation of flow centrifugation, filtration leukapheresis and dose of cells administered on the successful granulocyte transfusion therapy of septic dogs. (4) Evaluation of dose of autologous bone marrow tranfused in the survival of dogs receiving ablative chemotherapy. (5) Evaluation of schedules of administration of surgery, radiotherapy and chemotherapy on wound healing in mice.

3. Modeling Tumor Response to Therapy

The section has been actively engaged in the development and application of a generalized-Gompertzian model of tumor growth and response to therapy. The model is phenomenological in that only "inputs" (doses as functions of time) and "outputs" (tumor size) are modeled. Use of the model does not require the performance of difficult or impossible measurements (e.g. labeling indices, distribution of metabolites), nor does the model depend upon hypothetical mechanisms. The "data fitting" ability of the model has been documented in a variety of animal tumor systems with radiotherapy and single agent chemotherapy. More importantly, it has been demonstrated that the plausable assumptions of the model clearly imply that the conventional intensive induction with tapering maintenance therapy is sub-optimal in its anti-tumor effect. More effective schedules without additional toxicity have been identified. These results have very important potential clinical implications, particularly for surgical adjuvant studies.

A second very important application of this model is to the screening of drugs for anti-tumor activity in animal systems. We made the initial observation that in the B-16 melanoma of BDF_1 mice and the 13762 mammary carcinoma of Fisher rats the two parameters of the Gompertzian curve of tumor growth are perfectly correlated. This has now been confirmed in over a dozen other experimental tumors. An immediate implication of this is that the later portions of an unperturbed tumor growth curve for a particular animal is completely predictable based upon a few early measurements. Initial steps have been taken to apply this to the drug screening program. Our initial estimate is that the efficacy of the screening effort can be increased at least several fold by redesigning the experiments to start treatment after several early serial measurements of tumor size, and to use the estimated Gompertzian parameter as a co-variate for evaluation of the effect of the anti-cancer agent.

We have recently signed a contract with the Mount Sinai School of Medicine (principal investigator Larry Norton, M.D.) to test and collaborate in the further development of this model.

4. Multi-Institution Statistical Center Operations

The section serves as statistical center for the design, data collection, monitoring and analysis of selected major national clinical trials. We are currently engaged in two such projects. One is the ongoing studies of the Ovarian Cancer Study Group to evaluate staging procedures and treatments for patients with stages I-II ovarian cancer. We have devoted a considerable amount of effort this year to the development of protocols, forms, procedures and data systems for the new major national studies of the treatment of "resectable" cancer of the head and neck.

The head of the section is co-project officer for an international study of 1000 patients with non-Hodgkin's lymphoma. The major objective of the study is to compare the 8 histological classification systems used internationally in order to foster more uniform methods of reporting results.

5. Data Base System Development

The section designs, develops and maintains major data bases upon which its studies are based. It also consults with other programs within the division on the design of their major data bases. Major data bases that have been developed during this year include: (1) Hormonal receptor-treatment data base for breast cancer patients; (2) Data base of all clinical center patients with non-Hodgkin's lymphoma including histological classification by the five major systems; (3) Clinical data base of all NCI peritoneoscopies performed; (4) Data base of all Hodgkin's patients treated at the clinical center with combination chemotherapy; (5) Data base for national multi-institution study of "resectable" head-neck cancer.

In addition to these "specific" data bases, the section has designed and is developing a general data base system for all clinical trials performed intramurally in the Clinical Oncology Program. Disease specific initial data for all patients will be collected, as well as general and protocol specific data for each study. The system is general with a powerful table driven multi-field error-checking capability, and should decrease the need for future study-specific programming.

The section has also collaborated in the design of the protocol monitoring system for Phase I-II studies to be developed, maintained and utilized by the Cancer Therapy Evaluation Program. The section has also consulted on the statistical features of the PROMIS data system being implemented at the Baltimore Cancer Research Center.

6. Methodological Research

(i) Development of a method of sample size planning for clinical trials which compare a standard treatment to a conservative therapy. Such studies are becoming more common, but the usual test of the null hypothesis formulation is inappropriate.

(ii) Development of statistical methods for evaluating the relationship between treatment intensity and the induction of second malignancies. We have demonstrated that the frequently used "person-years" methods are invalid and may bias results against intensive treatments that prolong life.

(iii) Development of statistical methods for comparing treatments in carcinogenesis experiments where the endpoint is number of tumors per animal. Most previous statistical work has addressed carcinogenicity problems involving the proportion of animals who develop a tumor. For studies such as those investigating the effect of diet plus a known carcinogen, number of tumors per animal as a function of time is a more sensitive endpoint.

(iv) Statistical methodology for the analysis of cross-over designs was reviewed. Some restrictive assumptions (e.g., absence of carry-over effect) are not appropriate for many clinical trials in oncology (e.g., psychological carry-over in anti-emetic studies). It was shown that in some cases the restrictive assumptions could be relaxed and the methods generalized.

(v) Development of statistical methods for evaluating drug synergism using _in vitro_ cytotoxic assays.

(vi) Development of statistical methods and tables for the retrospective evaluating and block ranking of Phase II anti-cancer drugs based upon response rate. These methods permit the characterization of drugs as either active (e.g., >20% response rate), inactive (<10% response rate) or inadequately tested in a defined patient population with specified degrees of statistical reliability.

(vii) Development of a prospective two-stage patient accrual design for Phase II clinical trials. This design permits the use of early results to determine the number of patients studied in order to achieve a specified statistical reliability with a minimal number of patients.

(viii) Development of a generalization of Kendall's Tau for evaluating the association of an ordered parameter (e.g., age) with censored survival.

(ix) Evaluation of the effect of ignoring stratification factors in the analysis of clinical trials on the size and power of significance tests. Many clinical trials are over-stratified and the stratification factors are ignored in the analysis. The effect of this on study sensitivity was examined.

(x) Development of distribution free significance tests for randomized block designs comparing two treatments. These tests are particularly powerful for distributions consisting of a mixture of subpopulations of responsive and non-responsive patients.

(xi) Development of a mathematical measure of non-linearity for models to evaluate the degree of appropriateness of significance tests based on linear regression theory.

(xii) The general weighted least squares method for the analysis of categorical data was extended to the estimation of parameters for non-linear regression models.

(xiii) Development in collaboration with the Cancer Ther. Eval. Program of uniform criteria for evaluating tumor response by site for use by DCT clinical contractors conducting Phase II studies.

(xiv) Evaluation in collaboration with the Developmental Therapeutics Program of the design and analysis methodology used for the screening of new drugs in animal systems.

(xv) Development of methodology for relating levels of environmental pollutants to disease incidence using individual risk factor data based upon a sample survey.

Publications:

1. Kenady, D.E., Potvin, C., Simon, R. and Chretien, P.B.: *In vitro* effect of thymosin on T cell levels in cancer patients receiving radiation therapy. In *Control of Neoplasia by Modulation of the Immune System*, M.A. Chirigos (ed.), New York, Raven Press, 1977.

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3. Norton, L. and Simon, R.: Tumor size, sensitivity to therapy and the design of treatment schedules. *Cancer Treatment Reports* 61:1307-1317, 1977.

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8. Spence, R.J., Simon, R.M., and Baker, A.R.: Failure of immunotherapy with neuraminidase-treated tumor cell vaccine in mice bearing established methylcholanthrene sarcoma. *J. Nat. Cancer Inst.* 60:451-459, 1978.

9. Norton, L. and Simon, R.: New thoughts on the relationship between tumor growth characteristics and sensitivity to treatment. In Methods in Cancer Research - Cancer Drug Development, DeVita, V.T. and Busch, H. (eds). Academic Press, New York (in press).

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14. Simon, R.: Heterogeneity and standardization in clinical trials. In Proceedings of the E.O.R.T.C. Symposium on Controversies in Cancer Treatment, Staquet, M. and Sylvester, R. (eds). Masson Press (in press).

15. Rosenberg, S.A., Kent, H., Costa, J., Webber, B.L., Young, R., Chabner, B., Baker, A.R., Brennan, M.F., Chretien, P.B., Cohen, M.H., deMoss, E.V., Sears, H.F., Seipp, C. and Simon, R.: Prospective randomized evaluation of the role of limb sparing surgery, radiation therapy and adjuvant chemoimmunotherapy in the treatment of adult soft-tissue sarcomas. Surgery (in press).

16. Lippman, M.E., Allegra, J.C., Thompson, E.B., Simon, R., Barlock, A., Green, L., Huff, K.K., Do, H.M.T., Aitken, S.C. and Warren, R.: Lack of estrogen receptor is associated with an increased response rate to cytotoxic chemotherapy in metastatic breast cancer. N. Engl. J. Med. (in press).

17. Bender, R.A., Nichols, A.P., Norton, L. and Simon, R.M.: Lack of therapeutic synergism between vincristine and methotrexate in L1210 murine leukemia in vivo. Cancer Treatment Reports (in press).

18. Freeman, D.H., Jr., Makuch, R.W. and Stolwijk, J.A.J.: A strategy for the analysis of multiple area studies. Proceedings of the American Statistical Assoc., Social Statistics Section, 1977.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM-06402-08 CP

PERIOD COVERED
October 1,1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Tumor Growth Kinetics and Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Stanley E. Shackney, M.D., Senior Investigator CPB,DCT,NCI

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NCI/VA MOB
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LAB/BRANCH
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TOTAL MANYEARS: 3	PROFESSIONAL: 1	OTHER: 2

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Computer modeling studies of drug effects on cells *in vitro* are continuing. *In vitro* studies of two dose schedule optimization of hydroxyurea and cytosine arabinoside suggest that the optimum time for the second dose occurs when recruited cells are passing through mid-S. *In vitro* studies of adriamycin demonstrate proliferation dependent drug effect, without immediate suppression of DNA synthesis. Kinetic studies in Sezary's syndrome have shown that the skin is not the primary site of cell production, and that both the rate of cell production, and the rate of cell loss are high in this disease. Flow cytometry studies of the non-Hodgkin's lymphomas show that the large cell lymphomas have a high frequency of aneuploidy, and high proliferative rates in comparison with the small cell lymphomas.

1. Theoretical studies

1.1.1 The mammalian cell population kinetic simulator (MACKS)

The kinetic modeling system was expanded this year to include

a) the simulation of cell kill by DNA synthesis rate dependent drugs,
b) the capacity for exploring multidose, multidrug schedules,
c) a program for the analysis of DNA content distributions (FMF) and their relation to other kinetic parameters, in the steady state and under drug perturbation conditions.

Early drug modeling studies of dose interval optimization for two doses of a DNA synthesis rate dependent drug were found to be consistent with published observations in L1210 leukemia and ara-C and with our own *in vitro* studies. More definitive modeling studies along these lines will be carried out when the programming of the simulation package for growth retardation in sublethally damaged cells is implemented. This is a major modeling goal for the coming year.

1.1.2 A compartment model for Sezary's syndrome

Analysis of the Sezary's syndrome kinetic studies that were completed this year (see 3.1) indicated a compartment model with cell flow as shown:

A		B		C		
Primary site of cell production	→	Blood	→	Skin	→	Cell loss

A system of differential equations for a compartment model of this type was developed this year. Given known compartment sizes for compartments B and C, and compartment fluxes obtained from the kinetic data, the size of compartment A, and the rate of cell production in compartment A were calculated. Heuristic solutions suggest large tumor body burdens in compartment A ($\sim 2 \times 10^{11} - 1 \times 10^{12}$ cells) with high rates of cell production and cell loss (complete compartment turnover every 3-4 days). Definitive solutions will be obtained during the coming year using MLAB.

2. Experimental studies

2.1 Drug studies in Sarcoma 180 *in vitro*

2.1.1 Ara-C hydroxyurea schedule optimization studies

Schedule optimization studies employing two doses of hydroxyurea and/or ara-C were completed this year. Major findings included the following: maximum susceptibility to a second dose of drug occurred at the time when recovery in cell labeling intensity was maximal. This was 8-10 hours after a dose of hydroxyurea, and 13 hours after a dose of ara-C. This correlated well with the results of parallel flow cytometric studies (see 2.3.1). A manuscript describing these studies is in preparation.

2.1.2 Adriamycin studies

A dose response curve for cell survival by cloning was established following a 1 hour exposure to adriamycin in Sarcoma 180 *in vitro*. This was found to be multiphasic in log phase cultures. Below 1 μg/ml, cell survival plateaued at 40-60 percent of control, whereas above 1 μg/ml there was a sharp fall in cell survival to <10 percent. In plateau phase cells decreased cell survival was observed only at concentrations exceeding 1 μg/ml.

A dose response comparison of log and plateau phase cells after 24 hours of drug exposure is in progress.

In the coming year, the relation between adriamycin cell kill and DNA synthesis rate will be examined using the optimal spacing between two doses of hydroxyurea as an assay system for DNA synthesis rate dependence.

Attempts to explore the relation between cell kill and free radical formation in studies employing vitamin E and N-acetyl cysteine protection were not productive. The ethanol vehicle for vitamin E proved toxic to the cells, and N-acetyl cysteine did not afford protection at either high or low adriamycin doses in our *in vitro* system.

^{3}HTdR incorporation studies showed major differences between adriamycin and the DNA synthesis rate dependent drugs that we have examined so far. ^{3}HTdR incorporation following a 1 hour exposure to adriamycin did not fall immediately, as with ara-C and hydroxyurea, and may actually have increased initially. A late fall in ^{3}HTdR uptake was seen at 16 hours, with recovery by 24 hours in cell exposed to 0.5 μg/ml, and no recovery at 24 hours in cells exposed to 2 μg/ml. These observations correlated with flow cytometric studies performed in parallel (see 2.3.2). More detailed radioautographic studies are currently in progress.

In view of the relative resistance of Sarcoma 180 to adriamycin, cells were examined under fluorescence microscopy after exposure for 1 and greater than 24 hours. Adriamycin nuclear fluorescence was barely detectable. Similar observations in a CEM line at 1 and 4 hours showed greater nuclear fluorescence that in Sarcoma 180. These studies are being pursued with flow cytometric techniques (see 2.3.3).

2.1.3 Studies in Human Myeloma

A line of human myeloma cells which produces IgG has recently been acquired by our laboratory, courtesy of Dr. Benjamin Drewinko of the M.D. Anderson Hospital. Preliminary kinetic studies are being carried out on these cells at present. Studies employing fluorescent antibody markers will be used to:

a) study the kinetics of growth and differentiation in a lymphoid cell system, and

b) identify myeloma cells separately from normal marrow elements in clinical samples in flow cytometric studies.

This line will also be studied in Dr. Minna's laboratory using somatic hybridzation techniques.

2.2 Experimental mouse bone marrow studies *in vivo*

A radioautographic study of mouse bone marrow sections following treatment with hydroxyurea was initiated last year. The slide specimens could not be processed because of technical factors. This study was repeated during the past year. Marrow sections have been recut, and are currently being processed for radioautography.

2.3 Flow cytometry-experimental studies

2.3.1 Hydroxyurea and ara-C

Serial flow cytometric studies in Sarcoma 180 after a single dose of hydroxyurea and ara-C confirmed that cells synthesizing DNA at maximal rates were passing through the mid-S region of the DNA content distribution, and were the most susceptible to second doses of these agents at that time (see 2.1.1). The rapidity of data acquisition by this technique makes it feasible to use the hydroxyurea two dose optimization study as an assay system for DNA synthesis rate dependence (see 2.1.2).

2.3.2 Adriamycin studies

Serial studies on the effects of a 1 hour exposure of Sarcoma 180 to adriamycin were carried out in conjunction with our ^{3}HTdR labeling studies (see 2.1.2). The flow cytometric studies demonstrated conclusively that continued ^{3}HTdR incorporation was not related to DNA repair; the flow studies demonstrated unimpeded progression of cells through the mid-S region, with accumulation in the late S-G$_2$M region at 16 hours. Cells exposed to 0.5 μg/ml recovered by 24 hours, demonstrating a normal DNA content distribution, while cells exposed to 2 μg/ml remained in the late S-G$_2$M region. These observations proved of inestimable value in tying together the ^{3}HTdR and cell survival studies, and in interpreting the differences between adriamycin and other drugs.

The Coulter volume parameter demonstrated that the late S-G$_2$M were larger than usual suggesting that persistent adriamycin effect *in vivo* might be followed by relatively simple morphologic assessment.

2.3.3 Flow cytometric detection of DNA intercalated Adriamycin

In view of recent studies by Krishan that adriamycin quenches the fluorescence of DNA intercalating dyes such as propidium iodide and ethidium bromide these fluorescent staining techniques are currently being set up. Studies are being carried out to attempt to confirm Krishan's observations in Sarcoma 180, CEM and other cell lines at our disposal. If Krishan's observations are borne out, this may prove to be a useful technique for detecting active intracellular adriamycin and/or its active metabolites. This could be of value for predicting sensitivity to the drug clinically.

2.3.4 Cell sorting

The cell sorting capability of the Los Alamos instrument was made operational this year. Mixtures of latex microspheres of different sizes were sorted on the instrument; a rerun of the sorted sample showed a sorting efficiency exceeding 60% and sorting purity approaching 95%. Although some improvement is to be expected as more experience is gained, particularly regarding sorting efficiency, these results are well within performance specifications for the instrument.

Similar studies were carried out with mixtures of cells of different flourescence staining characteristics, and similarly good separation was achieved on the sorter.

2.3.5 Technical aspects of specimen processing

An aging effect has been observed in old fixed clinical samples with respect to mithramycin flourescence intensity. We have, therefore, taken care to process old samples with standards of comparable age. This has proven satisfactory in most instances, as corroborated by internal diploid reference cells.

Pursuant to a report by Barlogie *et al.* that brief pepsin digestion improves the reliability of staining of fixed specimens, we have tried this enzyme on several of our clinical specimens. Our limited experience to date suggests that pepsin counters this aging effect, and in addition, is an excellent disaggregating agent. Pepsin treatment does not appear to affect the Coulter signal adversely.

2.3.6 The T lymphocyte as a reference standard

A paper describing the T lymphocyte as a diploid reference standard in flow microfluorometry was completed this year and has been submitted for publication.

2.3.7 Human myeloma

Studies exploring the relation between protein content, IgG production and cell proliferation are being undertaken in our newly acquired human myeloma cell line (see 2.1.3).

Clinical studies

3.1 Sezary's syndrome

3.1.1 Pulse labeling studies

The study of two patients with Sezary's syndrome given pulse ^{3}HTdR was completed this year. A manuscript describing this work is nearing completion and will be submitted soon for publication. Computer modeling studies of these data indicate a large compartment of primary cell production with a high turnover rate (see 1.1.2). Clinical and pathologic findings would suggest that lymph nodes may be the primary site of cell production, and that Sezary's syndrome is a true lymphoma of T cell origin with prominent cutaneous manifestations. During the coming year serial studies of lymph nodes in patients with Sezary's syndrome will be carried out in collaboration with Dr. Bunn to see if they are, indeed, the primary site of cell production.

3.1.2 Sezary's syndrome, long-term ^{3}HTdR infusions

Analysis of the peripheral blood in one patient with Sezary's syndrome given a 20-day infusion is now complete. The slides from the peripheral blood of a second patient were unsuitable for analysis for technical reasons. Reserve slides are available and will be processed this year.

3.2 Tumor doubling times in small cell carcinoma of the lung

A paper describing tumor doubling times in small cell carcinoma of the lung has been accepted for publication in the journal Cancer. A major conclusion is that the long doubling times in small cell carcinoma suggest a long subclinical latent period between apparent complete response and tumor recurrence, especially when log kill is small. Cure should not be entertained in patients with disease free intervals of less than 4-5 years.

3.3 Kinetic studies in small cell carcinoma

Systemic ^{3}HTdR studies of the baseline kinetics of small cell carcinoma of the lung are planned in the coming year in collaboration with Dr. Bunn.

3.4 Flow Cytometry--clinical studies

3.4.1 Non-Hodgkin's lymphomas and leukemias

A total of over 70 specimens of lymph node, spleen, marrow and peripheral blood from patients with lymphomas and lymphoma/leukemias have now been analyzed by dual parameter flow cytometry. It is anticipated that analysis of another 30-40 specimens will be completed this summer.

While a definitive analysis of the data is premature at this point, provisional conclusions to date include the following:

1) Within a given lymphoma, the largest cells (including the largest G_1 cells) are the most rapidly proliferating. This indicates a strong correlation between kinetics and morphology.
2) Generally, the large cell lymphomas have more S and proliferate more rapidly than the small cell lymphomas.
3) The frequency of aneuploidy is higher in the large cell lymphomas than in the small cell lymphomas. Where present, aneuploidy, together with Coulter volume, permits the separate analysis of data from normal and neoplstic cells in the same specimen.

3.4.2 Ploidy of human tumors

A limited number of new effusions from solid tumors was accrued over the past year. Many of these proved to be non-neoplastic effusions. It is anticipated that a survey of 80-100 specimens will be feasible over the coming year.

3.4.3 Combined FCM and ^{3}HTdR labeling studies of diffuse large cell lymphomas

By agreement with the Medicine Branch, cells from fresh surgical specimens of the diffuse lymphomas will be processed by dual parameter FCM and HTdR labeling over the coming year. The first specimen obtained under this agreement has been processed without problems.

3.4.4 The monitoring of blastic transformation in CML

There have been numerous reports detailing the use of cytogenetics to predict blast crisis in CML months before clinical manifestation appear. We have observed aneuploidy in the peripheral blood of one patient with CML which led us to initiate a project to follow individual CML patients serially over time to see if FCM is of value in predicting blastic transformation.

3.4.5 Serial studies of drug perturbation

Serial multiparameter FCM studies in patients receiving single agents continue to be of high priority. Adriamycin is of particular interest. A patient with breast carcinoma and a pleural effusion who has failed CMF and is currently on a hormone protocol has been identified as a potential cadidate for such a study. Conversations have been held with Dr. Levine and Dr. Young to explore the feasibility of such studies in other patients over the coming year, possibly with other drugs.

Publications:

Shackney, S.E., McCormack, G.W. and Cuchrual, G., Jr. Growth Rate Patterns of Solid Tumors and Their Relation to Responsiveness to Therapy: An Analytical Review. Ann. Int. Med. 88: July, 1978.

Shackney, S.E., Cohen, M.H., Bunn, P.A., Jr., Ihde, D.C. and Minna, J.D. The Application of Principles of Cell Kinetics in the Design of Treatment Regimens for Small Cell Carcinoma of the Lung. In Lung Cancer, Current Trends in Treatment, (Muggia, F.M. and Rosencweig, M., Eds.) Raven Press.

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Cohen, M.A., Fossieck, B.E., Jr., Ihde, D.C., Bunn, P.A., Jr., Matthews, M.J., Shackney, S.E. and Minna, J.D. Chemotherapy of Small Cell Lung Cancer. Results and Concepts, In Lung Cancer Current Trends in Treatment, (Muggia, F.M. and Rosencweig, M., Eds.) Raven Press.

Shackney, S.E. The Orderliness of Cell Proliferation and Cell Differentiation in Relation to Kinetic Heterogeneity in Mouse Bone Marrow. In Cold Spring Harbor Symposium on Differentiation of Normal and Neoplastic Hematopoietic Cells. Clarkson, B., Marks, P.A., and Till, J., Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1978.

Bunn, P.A., Jr., Ihde, D.C., Cohen, M.H., Shackney, S.E. and Minna, J.D. Streptozotocin in Advanced Small Cell Bronchogenic Carcinoma: An Ineffective Nonmyelosuppressive Agent. Cancer Treatment Rep. 62: 479-482, 1978.

Shackney, S.E., Erickson, B.E., Jr. and Lincoln, T.L. Dual Parameter Flow Microfluorometric (FMF) Studies of the Proliferative Characteristics of the Non-Hodgkin's Lymphomas. In Proceedings of the Third International Symposium on Pulse Cytophotometry, Vienna, 1977. Lutz, D., eds., European Press, Medikon, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06510-02-CP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Mechanisms of Tissue Toxicity Caused by Adriamycin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles Myers, M.D.	Senior Investigator	CPB,DCT,NCI
OTHER:	Harold Londer, M.D.	Clinical Associate	CPB,DCT,NCI
	James Doroshow, M.D.	Clinical Associate	CPB,DCT,NCI
	Gershon Locker, M.D.	Clinical Associate	CPB,DCT,NCI

John Baldinger, 3rd yr. Med. Student, Vanderbilt University School of Medicine and CO-STEP Participant, Summer of 1978

COOPERATING UNITS (if any)

Robert Young, M.D.	Medicine Branch	DCT,NCI
William McGuire, M.D.	Medicine Branch	DCT,NCI
John Gottdiener, M.D.		NHLBI

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5	4	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. Adriamycin Cardiotoxicity: (Myers, Eliot, Brooks, Locker, Doroshow, McGuire)

This laboratory has recently proposed that adriamycin cardiotoxicity results from free radical generation during drug metabolism. The basic observations supporting this contention have been published in Science 197: 165 (1977).

We are currently examining the endogenous defenses of cardiac tissue against free radical damage to see what role they might play in the clinical pattern of cardiotoxicity. Finally, we are actively engaged in the design of a clinical trial testing the ability of various free radical scavengers such as Vitamin E to ablate adriamycin cardiac toxicity.

1. Adriamycin Toxicity

Doctors Doroshow and Locker are examining the role of free radicals in the etiology of adriamycin toxicity. Studies have evaluated the effect of manipulations of the seleno-enzyme glutathione peroxidase on murine cardiac and hepatic lesions due to adriamycin. We have found that adriamycin causes dose-dependent drop in cardiac glutathione peroxidase activity and that in the presence of a dietary deficiency of selenium and vitamin E, adriamycin toxicity is significantly enhanced.

We have also investigated the role of thiol compounds *in vivo* as agents capable of protecting against adriamycin toxicity. N-acetyl cysteine given orally has been found in this respect to significantly reduce adriamycin cardiotoxicity without antitumor activity.

2. Adriamycin Pharmacokinetics

Studies are underway to examine the relationship between clinical perturbations of hepatic or renal function and adriamycin disappearance and metabolism in our patients. These investigations will also explore the effect of chemotherapeutic drug combinations containing adriamycin on adriamycin pharmacokinetics. We have also studied the effect of hyperthermia on adriamycin pharmacology.

3. Specific Sites of Cardiac Biochemical damage following adriamycin

With Helen Eliot, we are examining the specific omponents of the cardiac cell damage following adriamycin. Given the working hypothesis that the damage proceeds via a free radical mechanism, it is possible to predict that such endogenous compounds as coenzyme Q10 and membrane phospholipids should be destroyed to yield identifiable oxidized products. We are in the process of studying these changes as they might provide important insight as to why the lesions can progress for months after the last dose of adriamycin. We hope these studies will provide a biochemical explanation for the cumulative nature of adriamycin cardiac toxicity.

Publications:

Myers, C.E., McGuire, W.P., Liss, R.H., Ifron, I., Grotzinger, K.; Young, R.C.: Adriamycin: The Role of Lipid Peroxidation in Cardiac Toxicity and Tumor Response, *Science* 197: 165 (1977).

Myers, C.E., McGuire, W.P., and Young, R.C.: Adriamycin: Amelioration of Toxicity by α-Tocopherol. *Cancer Treatment Reports*, Vol. 60, No. 7: 961-962, July, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06511-02-CP

PERIOD COVERED October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Molecular Pharmacology of Cyclic Nucleotides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Dr. Chi-Ming Liang, Visiting Fellow CPB,COP,DCT,NCI

OTHER: Dr. Vernon Wong - Dept. of Ophthalmology, Georgetown University
Dr. Gerald Chader - Lab. of Vision Research NEI
Dr. Mary O. Lim, Pediatric Medicine NICHD

Stay-In-School Program: Howard Schwartz - University of Maryland
(July 1, 1977 to December 31, 1977)
Brenda Waller (February 12, 1978 to)
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A major goal of research in cyclic nucleotides is to study the biochemistry of enzymes, proteins and compounds which involve in synthesis and degradation of the cyclic nucleotides and to link this knowledge to the understanding of the physiological functions in given tissues. The activity of cyclic AMP and cyclic GMP phosphodiesterase (PDE) and the protein activator of cyclic AMP phosphodiesterase in various anatomic and subcellular fractions of the bovine eye were studied. A heat-stable, non-dialyzable inhibitory factor of cyclic nucleotide phosphodiesterase was detected in and partially purified from bovine retina. The factor appears to be a protein. A small dialyzable molecular phosphodiesterase inhibitor was also isolated from bovine brain. This inhibitor is not a protein since it is not hydrolyzed by different proteolytic enzymes. The activities of cyclic nucleotide PDE and protein activator were measured in Brown-Pearce (rabbit) carcinoma before and after methotrexate perfusion. The differences in enzyme and activator activities were observed before and after the treatment of methotrexate. An abnormality in retinal cyclic nucleotide metabolism in inherited rod-cone dysplasia of Irish setter dog was observed.

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(Rev. 10-76)

In the bovine eye, cyclic GMP hydrolysis was 1.6 to 12-fold faster than hydrolysis of cyclic AMP in the subcellular fractions of the retina and in the precipitate of the rod outer segment. An opposite pattern was seen in the bovine lens, where the hydrolysis of cyclic AMP occurred 17 and 169 times faster than that of cyclic GMP in the supernatant and precipitate of lens, respectively. The activity of cyclic AMP phosphodiesterase was not affected by EGTA in any fractions except in the retinal supernatant suggesting that the phosphodiesterase exists primarily as a Ca^{2+}-independent, activator-independent form. However, the protein activator of cyclic AMP phosphodiesterase existed in all fractions examined. A complex kinetic pattern was observed for both cyclic AMP and cyclic GMP hydrolysis by the 105000 x g lens supernatant. The Michaelis constants for both cyclic AMP (1.3×10^{-6} and 9.2×10^{-6} M) and cyclic GMP (1.04×10^{-6} and 1.22×10^{-5} M) appeared to be similar.

The protein inhibitor from bovine retina effectively inhibits the Ca^{++}-independent phosphodiesterase from several sources, including bovine retina, bovine rod outer segment, and a human lymphoblastic leukemia cell line, indicating lack of tissue and species specificity. The inhibitory effect on the phosphodiesterase of a human lymphoblastic leukemia cell line was partially reversed by the bovine brain protein activator and was not altered by Ca^{++}.

The chromatography of molecular sieving, cation and anion exchange, high pressure liquid and thin layer and spectrophotometric measurement were used to isolate and identify a small molecule inhibitor of phosphodiesterase from bovine brain. This inhibitor was confirmed as hypoxanthine. Since cells, if deprived of oxygen, secrete large amounts of hypoxanthine and inosine, the effect of these compounds on phosphodiesterase was evaluated.

For the activator dependent form of phosphodiesterase, the ID_{50} of hypoxanthine, 5'-inosine monophosphate (IMP) and inosine were 2.65 mM, 2.3 mM and .04 mM respectively which were 5-30 folds less than theophylline (ID_{50} = 0.082 mM). The inhibitions were not reversed by the addition of Ca^{++}. The activator independent form of phosphodiesterase was also inhibited by these compounds with ID_{50} 1.85 mM (hypoxanthine), 2.3 mM (IMP), and 0.3 mM (inosine).

Although the Ca^{++}-dependent form of cAMP PDE was found only in the supernatant fraction, the protein activator of cAMP PDE was present in all fractions derived from the Brown-Pearce carcinoma; the perfused Brown-Pearce carcinoma appeared to have a lower protein activator level. Brown-Pearce carcinoma perfused with methotrexate showed a lower level of cAMP PDE in homogenate and supernatant fractions but a higher activity of cAMP PDE in nuclear fraction. Elevation of cGMP PDE was observed in all fractions, except homogenate, derived from methotrexate treated Brown-Pearce carcinoma. The ratio of cAMP PDE to cGMP PDE was lower in perfused than in non-perfused carcinoma.

Irish setter dogs are known to carry an autosomal recessive mutation that causes retinal degeneration in homozygous animals. Affected Irish setter animals (1-12 weeks of age) were studied biochemically to assess whether cyclic nucleotide metabolism was normal. Unaffected heterozygate Irish setters of similar age were used as control animals.

The level of cyclic GMP in the retinas of affected dogs is about 10 times greater than in control retinas. The activity of cyclic GMP PDE in the retinas of affected dogs is about 3-4 times lower than in control retinas while the level of cyclic AMP PDE showed the same for both affected and control retinas. The activator activity of cyclic GMP PDE was also lower in diseased retinas than in control. The activator activity of cyclic AMP PDE remained about the same for the diseased and control retinas. Addition of exogenous activator to the diseased retinas increased the cyclic GMP PDE activity. However, the exogenous activator did not affect the cyclic GMP PDE of control retinas. The results suggest that the diseased retinas either contain insufficient activator for the maximal activation of PDE or have altered cyclic GMP PDE which required more activator to be fully activated.

Cyclic nucleotides, cyclic nucleotide PDE and protein activator of PDE were studied in cultured skin fibroblasts in a case of dysplastic intradermal calcification. The level of cyclic AMP is lower in the diseased cultured cells than in the control. Cyclic AMP PDE was markedly elevated (mean 708 pmoles/mg min.) compared to controls (mean 212 pmoles/mg min.).

Publications:

Liu, Y.P., Wong, V., and Chabner, B.A.: Cyclic nucleotide phosphodiesterase and protein activator in human cancer cell lines and Brown-Pierce carcinoma. J. Natl. Cancer Inst. 59: 1605-1609, 1977.

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Liu, Y.P., and Schwartz, H.S.: Protein Activator of Cyclic AMP Phosphodiesterase and Cyclic Nucleotide Phosphodiesterase in Bovine Retina and Bovine Lens: Activity, Subcellular Distribution and Kinetic Parameters. Biochem. Biophys. Acta. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06512-02-CP

PERIOD COVERED October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Molecular Toxicology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Clinical Pharmacology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	7.0	7.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Progress on each individual subproject is described in detail herein. Major areas of progress are summarized as follows: 1) 3-Methylfuran and carbon tetrachloride have been shown to be metabolized in vivo to highly reactive metabolites that cause necrosis of pulmonary Clara cells, emphasizing the importance of our earlier discovery that Clara cells are an important site of xenobiotic-metabolizing enzymes in lung. 2) The validity of a mechanism of acute renal tubular necrosis involving metabolic activation of a parent drug to an alkylating metabolite *in situ* by cytochrome P450-dependent enzymes has been demonstrated. 3) The importance of species and age differences in the specificity of target organ alkylation and toxicity by a metabolically-activated alkylating agent has been demonstrated. 4) Immunochemical studies suggest that cytochrome b_5 may play an important role in the metabolism of certain types of xenobiotics in the lung. 5) The glandular gastric mucosa of rats has been found to contain exceedingly high concentrations of reduced glutahtione (GSH), raising the question of its potential physiologic significance as well as havin important implications for gastric carcinogenesis. 6) A new clinical HPLC ass for 5-fluorouracil has been developed.

PHS-6040
(Rev. 10-76)

Objectives:

The primary goals of the research program in Molecular Toxicology are a) to elucidate fundamental chemico-biologic interactions underlying target organ and target cell cytotoxicities and carcinogenicities by chemical agents, and b) to provide consultation and expertise in analytical methodologies required by clinical research programs of the Clinical Pharmacology Branch, NCI.

Methods:

A wide variety of instrumentation and techniques are employed in these investigations. Metabolic and dispositional studies utilize traditional methodologies, and instrumental techniques including GC-mass spectrometry and high-pressure liquid chromatography. Toxicologic studies employ biochemical measures of cellular alterations as well as morphologic measures such as high-resolution light microscopy, electron microscopy and autoradiography.

Descriptions of subprojects; major findings and planned courses of projects:

1. Mechanism of Nitrofurantoin-induced lung disease. Nitrofurantoin (NF), a widely used urinary tract antibiotic, is one of the drugs most commonly reported to cause serious lung disease in patients. We have developed an animal model for nitrofurantoin-induced pulmonary toxicity and have found that the reaction resembles an exaggerated form of pulmonary oxygen toxicity. An oxygen-enriched atmosphere synergizes the toxic response to NF. In vitro studies indicate that the superoxide radical is generated during metabolism of NF by lung enzymes. Moreover, feeding a vitamin E-deficient diet, or a diet high in polyunsaturated fat, both increase susceptibility to NF-induced lung lesions. These results suggest that NF causes lung lesions by stimulating the production of superoxide in vivo, and that superoxide may cause the peroxidative destruction of essential lipids in the lung.

A major study of the in vivo distribution and metabolism of ^{14}C-NF in control and vitamin E-deficient rats is underway to determine if vitamin E enhances nitrofurantoin toxicity by an effect on NF disposition rather than by loss of an antioxidant activity. Studies to develop a model of pulmonary fibrosis with NF have also been undertaken in collaboration with Dr. Jerold Last, University of California School of Medicine, Davis, California.

2. Effects of salts of cobalt and other divalent metals on glutathione biosynthesis. We recently discovered that administration of cobaltous chloride to a variety of animal species causes striking increases (100-200%) in concentrations of reduced glutathione (GSH) in several tissues, including liver, lung, kidney and ileum. Several other divalent metal salts also had this action, but were generally less effective than cobalt. Since many electrophilic chemicals or metabolites are detoxified in vivo by conjugation with GSH, the enhanced GSH availability caused by metallic ions has important toxicologic implications. Moreover, the striking effects of cobalt salts on GSH appears to offer an important new tool for the study of GSH biosynthesis in vivo and further studies will be undertaken to explore this possibility.

3. Cellular site of drug-metabolizing enzymes in lung: implications for toxicity and carcinogenesis. Recent studies in our laboratory indicate that nonciliated pulmonary bronchiolar cells (Clara cells) are a site of cytochrome P450-dependent monooxygenase activity in lung, a finding that has important implications for the pathogenesis of chemically-induced pulmonary disease. Cytotoxic, and/or carcinogenic metabolites may be produced in these cells; the Clara cell may therefore be an especially susceptible target cell for toxins or carcinogens that are metabolically activated by cytochrome P450-dependent pathways. Studies with 3-methylfuran and carbon tetrachloride, which are described below, are consistent with this view. In continuing studies we hope to further elucidate the role of the Clara cell in the pulmonary metabolism of xenobiotics and also to explore the possibility that other pulmonary cell types contain xenobiotic-metabolizing enzymes, with perhaps different substrate specificities. To facilitate these investigations we are developing new systems for the study of xenobiotic metabolism in isolated lung cells, lung slices, isolated perfused lungs, and in "reconstituted" enzyme systems containing highly purified forms of cytochrome P450 isolated from lung. Some of the latter studies will be pursued in collaboration with Dr. John R. Bend and coworkers, Laboratory of Pharmacology, NIEHS, NIH, Research Triangle Park, N.C.

4. Role of cytochrome b_5 in the metabolic activation of 4-ipomeanol (IPO) by lung microsomes. In vitro experiments revealed that the V_{max} per nanomole of P-450 for formation of an alkylating metabolite of IPO was about 10-20 times greater in pulmonary than in hepatic microsomes. The high rate of lung microsomal alkylation could not be due to a deficiency in lung microsomal detoxication pathway(s), such as epoxide hydratase, since the ratio of metabolites covalently bound/total metabolites formed was about 1/2 for pulmonary and 1/3 for hepatic microsomes. Although CO inhibited the activation, an antibody prepared against purified cytochrome b_5, which was inactive against TPNH/cytochrome c reductase, almost completely inhibited the TPNH-supported metabolism of IPO by pulmonary, but not hepatic, microsomes. Preincubation of the antibody with purified cytochrome b_5 abolished its inhibitory effect on lung microsomal metabolism of IPO, suggesting that the inhibition was due to a specific effect of the antibody on microsomal cytochrome b_5. These results suggest that IPO activation involves a two-electron transfer in which transfer of the second electron via cytochrome b_5 is rate-limiting in lung microsomes, a factor which would possibly account for the pulmonary cellular specificity of IPO, and possibly other metabolically activated chemicals, in vivo. We plan to further probe this question using "reconstituted" monooxygenase systems using highly purified cytochrome P450 and b_5 and other essential components.

5. Pulmonary metabolism of xenobiotics in birds, species which lack pulmonary Clara cells. Avian lungs lack the nonciliated and ciliated bronchiolar epithelium typical of lungs of other mammalian species. Based on this morphologic difference, and our recent finding that nonciliated pulmonary bronchiolar cells are a site of cytochrome P450-dependent xenobiotic-metabolizing enzymes in other species, we are studying xenobiotic metabolism by avian lungs in vitro and in vivo. Compounds of particular interest in these studies are 4-ipomeanol (IPO), an agent that is preferentially metabolized to a highly toxic alkylating agent in nonciliated pulmonary

bronchiolar cells of other species, and benzo(a)pyrene (BP), a ubiquitous environmental pulmonary carcinogen.

6. Pulmonary Clara-cell toxicity by carbon tetrachloride. Carbon tetrachloride (CCl_4) is an extensively studied model hepatotoxin that requires hepatic mixed-function oxidase (MFO)-mediated activation to produce liver necrosis. Others have shown that oral administration of CCl_4 causes decreases in lung MFO activity, but were unable to associate this biochemical lesion with a specific pulmonary cellular lesion. Therefore, we have examined the *in vivo* effects of CCl_4 on pulmonary Clara cells. Oral administration of 2.5 ml/kg of CCl_4 to mice and rats caused striking decreases in lung microsomal cytochrome P450, benzphetamine demethylase activity, and in the pulmonary microsomal metabolism of 4-ipomeanol. Previous studies indicated that 4-ipomeanol is selectively metabolized by MFO enzymes localized in Clara cells. Histologic examinations of lungs of CCl_4-dosed animals revealed morphologic changes of Clara cells including lipid accumulation, dilation of endoplasmic reticulum, and cellular necrosis. These results suggest that pulmonary Clara cells are susceptible to CCl_4-induced injury due to their unique capacity to metabolically activate the chemical, and are consistent with, and further support, our previous work indicating the Clara cell as a locus of cytochrome P450-dependent enzymes in lung. This project is essentially complete and no further studies are planned.

7. Metabolism and toxicity of 3-methylfuran. A recent report from the Naval Research Laboratory suggested that a major air pollutant during photochemical smogs in Washington, D.C., and probably other cities, is 3-methylfuran (3MF). It has been speculated that 3MF is derived from the photodegradation of terpenoid compounds produced by deciduous vegetation. Based on our previous studies showing profound pulmonary toxicities by 3-substituted furan derivatives, we have studied the metabolism and toxicity of 3MF.

Analogous to 4-ipomeanol, 3MF is metabolized *via* cytochrome P450-dependent oxidases to a highly toxic alkylating agent in pulmonry bronchiolar cells. This occurs when 3MF is given ip, or by inhalation. In continuing studies we are further characterizing the metabolism and toxicologic properties of 3MF. A long-term toxicity study and carcinogenesis assay on 3MF is being undertaken in collaboration with Dr. Henspeter Witschi, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

8. Biochemical and morphological correlates in drug-induced lung injury. Although our previous studies have demonstrated the participation of reactive metabolites in chemically-induced lung injury, the precise mechanisms of chemical/tissue interactions in the pathogenesis of the lesions has not been elucidated. In this project we are approaching these questions using a combination of biochemical measures coupled with morphologic techniques, including high-resolution light microscopy, electron microscopy, and autoradiography. Through careful study of the time courses and dose-dependencies of the metabolism of pulmonary cytotoxins, the site of cellular interactions of reactive metabolites, changes in selected cellular biochemical parameters, and morphologic alterations, we hope to determine the

critical chemico-biologic events leading to cell death. Compounds currently under study in this project include 4-ipomeanol, α-naphthylthiourea, nitrofurantoin, and 3-methylfuran.

9. Role of reduced glutathione (GSH) as a modulator of drug-induced pulmonary toxicities. Other investigators have shown that liver GSH can combine with highly reactive electrophilic drug metabolites to form non-toxic excretable derivatives, and it can thereby protect the liver from potentially hepatotoxic metabolites. We have recently found that GSH may play a similar "protective" role against the lung lesions induced by an electrophilic metabolite of 4-ipomeanol. These studies are being extended to examine the hypothesis that GSH may play a general protective role against a variety of lung lesions induced by direct alkylating agents as well as alkylating metabolites.

10. Species and strain differences in tissue alkylation and toxicity by 4-ipomeanol: predictive value of covalent binding in the study of target organ toxicity. In this project we are exploring the possibility that species and strain differences in target organ alkylation and toxicity by 4-ipomeanol (IPO) can be utilized to help elucidate metabolic and dispositional factors determining the target organ specificity of metabolically-activated cytotoxins. Previous studies have demonstrated highly preferential alkylation of rat lungs *in vivo* by a metabolite of IPO. The covalently bound IPO metabolite was localized in the pulmonary bronchioles; pulmonary bronchiolar necrosis was the initial lung lesion produced by IPO. We have found a similar pattern of target organ selectivity for IPO in the rabbit and guinea pig. No renal or hepatic lesions were seen in any of these species. In contrast, in addition to pulmonary bronchiolar lesions, IPO produced striking renal cortical necrosis in adult mice and centrilobular hepatic necrosis in hamsters. IPO metabolite was covalently bound preferentially in the kidneys of adult mice and in the liver of hamsters. For a given dose of IPO the tissue levels of covalently bound IPO metabolite were much lower in the hamster than in any other species. Correspondingly, the hamster was much less susceptible to IPO toxicity than any other species. Similarly, with different strains of rats and mice, there was a positive correlation between the level of target organ alkylation by an IPO metabolite and the relative level of susceptibility to IPO-induced toxicity. These studies indicate that the easily determined index of *in vivo* covalent binding may have predictive utility in screening for both target organ selectivity and potency of toxins or drugs producing lesions *via* alkylating metabolites.

11. Role of renal metabolism in chemical-induced renal necrosis. Administration of the furan derivative, 4-ipomeanol (IPO), to adult mice resulted in the preferential alkylation of the kidneys by an IPO metabolite. Correspondingly, unlike other species we have studied, the mouse responded to IPO with a striking renal cortical necrosis, in addition to pulmonary bronchiolar necrosis. Autoradiographic studies demonstrated that the covalently bound IPO metabolite was highly localized in those renal cortical tubules which became necrotic. *In vitro* studies demonstrated that IPO was converted to an alkylating metabolite *via* cytochrome P-450-dependent mixed-function oxidase activity present in mouse liver, kidney, and lung microsomes. Formation of an alkylating metabolite of IPO was greatly increased in liver

microsomes, but not renal microsomes, from C57BL/6J mice pretreated with 3-methylcholanthrene (MC). In vivo hepatic alkylation by an IPO metabolite was markedly elevated in the induced mice, while renal alkylation was markedly decreased. IPO produced striking centrilobular hepatic necrosis in the induced mice, but much less intense renal necrosis, as compared to non-induced controls. In contrast, MC pretreatment had no significant effect on target organ alkylation and toxicity by IPO in "noninduceable" DBA/2J mice. These results support the view that the highly reactive metabolite of IPO that causes renal necrosis in mice is actually formed within the target tissue, and not transported to the kidney after formation in the liver. These studies establish the validity of a mechanism of renal injury involving in situ metabolic activation, which may also apply to other agents subject to metabolic activation.

12. Age-related differences in target organ alkylation and toxicity. Recent studies indicate that the pattern of target organ alkylation and toxicity by 4-ipomeanol in the mouse is age-dependent. Young mice show high pulmonary alkylation and toxicity but low renal alkylation and no renal cortical necrosis. In contrast, adult mice show high levels of renal alkylation and renal cortical necrosis and relatively lower levels of pulmonary alkylation and bronchiolar necrosis. In continuing studies we are attempting to elucidate whether these striking differences are due to age-related metabolic and/or distributional factors. We believe these studies will have important general implications for studies of target organ toxicities and carcinogenicities by compounds requiring metabolic activation.

13. *In vitro* and *in vivo* studies of stable metabolites of metabolically-activated pulmonary toxins as an approach to the identification of highly reactive, lung-toxic metabolites. Highly reactive, toxic drug metabolites combine covalently with tissue macromolecules, rearrange spontaneously, or are further metabolized; thus, in most cases they cannot actually be isolated and identified. One alternative in identifying the chemical nature of these highly reactive species therefore is to carefully determine the characteristics of the secondary metabolites or rearrangement products, in order to permit a rational speculation as to the nature of the intermediates from which they were derived. We are currently applying this reasoning to the study of the stable metabolites, formed both in in vivo and in vitro systems, of 4-ipomeanol, nitrofurantoin and 3-methylfuran.

14. Evaluation of 4-ipomeanol (IPO) and other organ-specific alkylating agents for tumor-specific metabolism and cytotoxicity. The purpose of this project is twofold: a. to explore the question of the cell of origin of tumors, based on specific metabolic similarities with normal cellular precursors and b. to evaluate the potential antitumor properties of organ-specific and cell-specific alkylating agents. Of particular interest in this project is 4-ipomeanol (IPO), a furan derivative that is preferentially metabolized to an alkylating agent in nonciliated pulmonary bronchiolar epithelium. IPO therefore is potentially a unique tool for the study of tumors derived from the nonciliated bronchiolar epithelium. The potential cytotoxicity of IPO, and related compounds, to bronchiogenic pulmonary tumors is also under active investigation. Collaborators on these studies include Dr. Adi Gazdar, NCI-VA Medical Oncology Program, Veterans Adminstration Hospital, Washington, D.C., and Dr. Randolph McMurtry, Department of

Pathology, University of Colorado School of Medicine, Denver, Colorado.

15. Assay of 5-fluorouracil (FU) and 5-fluorodeoxyuridine (FUdR) in plasma at the low nanogram level using high-pressure liquid chromatography. Previously published methods for the quantitative determinations of plasma levels of FU and FUdR have either lacked sensitivity and specificity or have required sophisticated techniques such as gas chromatograph-mass spectrometry (GC-MS). We have devised a rapid, simple HPLC assay for FU and FUdR in plasma which has sensitivity equal to published GC-MS methods. The method involves a preliminary sample clean-up using anion exchange chromatography, followed by flow-programmed HPLC analysis. Radiolabeled internal standards (commercially available) are used to conveniently correct for extraction and measurement efficiencies. Plasma concentrations of FU or FUdR as low as 5×10^{-8} M can be readily measured with the method. The applicability of the method to clinical use has been verified by successful preliminary studies of FU in two patients, one receiving FU by intrahepatic infusion, and another as an IV bolus.

16. High levels of reduced glutathione in glandular rat stomach; possible implications. The demographic patterns of incidence of gastric adenocarcinoma suggest that this tumor may be carcinogen-related. Unfortunately, many attempts to develop experimental models of carcinogen-induced gastric cancer in animals have failed, or cancers have developed only in the squamous portions of rodent stomachs. The glandular segments of rodent stomachs are most analogous anatomically to the human stomach. We have found exceedingly high (5-10mM) levels of the nucleophilic tripetide, reduced glutathione (GSH), in the glandular portions of rat stomachs. Glandular gastric GSH levels nearly equalled the levels in liver and far exceeded levels in all other tissues tested. GSH levels in the esophagus, squamous portion of the stomach, ileum, and colon were only 15-30% of the levels of the glandular stomach. Glandular stomach GSH varied diurnally; it was highest in the afternoon and lowest at night. Starvation caused a decrease in glandular gastric GSH as did pretreatment with diethylmaleate. In animals stressed using the cold-restraint method, both hepatic and glandular gastric GSH levels were decreased by 30-40%. These findings suggest that, by virtue of the high levels of GSH, the glandular stomach may be relatively resistant to highly electrophilic, carcinogenic chemicals, or metabolites, which can covalently complex with GSH to form less reactive, noncarcinogenic conjugates. The finding of stress-induced decreases in GSH suggests a mechanism whereby stressors could modulate susceptibility to chemical carcinogens. Finally, the high concentrations of gastric GSH raise the additional question of its significance to normal gastric physiology. Further studies will be undertaken to address these questions.

17. Mechanism of action of hexamethylmelamine(HMM). Based on its antitumor activity profile, we suspect that metabolic activation of HMM may be required for the expression of its antitumor activity and may underlie its particular pattern of antitumor specificity. Preliminary *in vitro* studies support this hypothesis and additional studies both *in vitro* and in tumor test systems *in vivo* will therefore be undertaken to further explore the question.

Significance to biomedical research and the program of the institute:

The potential utility of existing cancer chemotherapeutic agents is frequently hindered by dose-limiting toxicities to target organs such as the gastrointestinal tract, bone marrow, liver, kidney and lung. Moreover, many antitumor agents in current use are also, in themselves, potent carcinogens. Thus, as the longer-term survival of patients on antitumor drug therapy becomes a reality, paradoxically, the problems of drug-induced secondary cancer will assume greater significance.

The elucidation of molecular mechanisms whereby cytotoxic and/or carcinogenic chemicals exert their biologic effects hopefully will contribute to the improved usage of existing chemotherapeutic agents, and will help provide a more rational basis for the design of new drugs with optimal tumoricidal activity but minimal toxicity to normal tissues.

Publications:

Boyd, M.R.: Evidence for the Clara cell as a site of cytochrome P450-dependent mixed-function oxidase activity in lung. *Nature* 269: 713-715, Oct., 1977.

Boyd, M.R., Statham, C.N., Franklin, R.B., and Mitchell, J.R.: Pulmonary bronchiolar alkylation and necrosis by 3-methylfuran, a potential atmospheric contaminant derived from natural sources. *Nature* 272: 270-271, March, 1978.

Boyd, M.R., and Burka, L.T.: *In vivo* studies on the relationship between target organ alkylation and the pulmonary toxicity of a chemically reactive metabolite of 4-ipomeanol. *J. Pharmacol. Exp.* Therap.: in press, 1978.

Boyd, M.R., Osborne, B.A., Burka, L.T., and Wilson, B.J.: *In vitro* studies on the metabolic activation of the pulmonary toxin, 4-ipomeanol, by rat lung and liver microsomes. *J. Pharmacol. Exp. Therap.*: in press, 1978.

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Boyd, M.R.: Metabolic activation of toxic agents. In *Mechanisms in Respiratory Toxicology*. Eds. H. Witschi and P. Nettesheim, CRC Uniscience, in press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06513-02-CP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Molecular Pharmacology of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	David Roselle, Student Scientist, Bucknell University (Summer 1978)	
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COOPERATING UNITS (if any) Pediatric Oncology Branch; Surgery Branch
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Medicine Branch;
Veterans Administration Oncology Branch, Lab of Medicinal Chem.Biology

LAB/BRANCH
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SECTION

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4	3	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies are directed at understanding the quantitative and qualitative interactions of antitumor agents with target sites in tumor cells. Emphasis has been placed on understanding factors which determine response to antifolates, 5-fluoro-uracil, and the pineal hormone, melatonin at the cellular and biochemical level. In vitro uptake, binding to receptor sites, and cytotoxicity of these agents are examined in tissue culture systems as well as in the whole animal.

1. Studies of methotrexate binding to dehydrofolate reductase in L_{1210} cells have demonstrated reversibility of the drug enzyme complex and have defined an "off" rate which is 30 fold faster than the corresponding "off" rate of drug from highly purified dehydrofolate reductase. Further studies are underway to define the effective changes in cofactor concentration, cations, sulfhydril group modifications, and other factors on enzyme drug interaction.

2. The synergistic interactions of 3-deazauridine and cytosine arabinoside has been demonstrated in Sarcoma 180 cells. 3 deazaUR increases ara-CTP levels, and was shown to inhibit cytidine deaminase and as the monophosphate, deoxycytidine deaminase, two degradative steps in the ara-C pathway. The biochemical basis for synergism of these two agents is being further investigated in human and murine tumor lines.

3. We have obtained evidence for a new metabolite of methotrexate, 2,4 diamino N-10 methyl pteroic acid, which cross reacts with the radioimmunoassay antibody for methotrexate, leading to spurious elevation of drug levels determined by that method. Further attempts to isolate this metabolite and quantitate it in plasma and urine are underway.

4. Studies with the human breast cancer cell line ZR 75, grown in a defined, nucleoside free medium, have shown that methotrexate and fluoropyrimidines have synergistic inhibitory effects on cell growth at drug levels which exclusively block thymidylate synthesis. Further studies are underway to determine the ability of methotrexate to participate in a terniary complex with FdUMP and thymidylate synthetase.

5. Clinical pharmacology studies of phosphono-acetyl-L asparatic acid are proceeding in concert with a phase I trial. Drug levels are being determined by Dr. John Strong of the Laboratory of Chemical Pharmacology, and Dr. David Cooney of the Laboratory of Toxicology.

   Studies of hepatic clearance of 5-FU, administered by intrahepatic infusion, have begun, using a highly sensitive HPLC assay devised by Dr. Alan Buckpitt, which can detect less than 5×10^{-7} M 5-FU in plasma.

   Routine and investigative monitoring of plasma methotrexate levels is ongoing and under the direction of Dr. Charles Myers.

6. Studies of the physiologic action and antitumor effects of melatonin are progressing. A specific cytoplasmic receptor has been identified in gonadal tissue, liver and skin. It was also shown that melatonin suppresses induction of mammary carcinoma in rats by DMBA, while pinealectomy causes accelerated induction of this tumor.

7. Studies of allopurinol pharmacokinetics in man have been completed and have been accepted for publication (Clin. Pharm. & Therap., in press). A competitive binding assay for allopurinol and oxipurinol has been developed using xanthine oxidase as the binding protein. These results have shown that activation of allopurinol is not inhibited by the

presence of oxipurinol, raising questions as to the mechanism of conversion of allopurinol to oxipurinol. The conversion of allopurinol to oxipurinol by various modified forms of xanthine oxidase is being studied.

Publications:

Drake, J.C., Stoller, R.G. and Chabner, B.A.: Characteristics of the enzyme uridine-cytidine kinase isolated from a cultured human cell line. Biochem. Pharmacol. 26: 64-66, 1977.

Stoller, R.G., Hande, K.R., Jacobs, S., Rosenberg, S.A. and Chabner, B.A.: Plasma levels predict toxicity following high dose methotrexate infusion. N. Engl. J. Med. 297: 630-634, 1977.

Stoller, R.G., Myers, C.E. and Chanber, B.A.: Analysis of cytidine deaminase and tetrahydrouridine interaction by use of ligand techniques. Biochem. Pharmacol. 27: 53-59, 1978.

Hande, K.R., Chabner, B.A.: Pyrimidine nucleoside monophosphate kinase from human leukemic blast cells. Cancer Res. 38: 579-585, 1978.

Hande, K., Reed, E., and Chanber, B.A.: Allopurinol pharmacology in man. Clinical Pharmacology and Therapeutics, in press.

Cohen, M., Bender, R.A., Donehower, R., Myers, C.E., and Chabner, B.A.: Reversibility of high affinity binding of methotrexate in L1210 Murine leukemia cells. Cancer Res., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-03403-13-M

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

CLINICAL TRIALS AND MISCELLANEOUS CLINICAL INVESTIGATIONS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Robert C. Young, Chief, Medicine Branch, COP, DCT, NCI
Others: Senior Investigators, MB, COP, DCT, NCI: Richard A. Bender, Bruce A. Chabner, Tom Anderson, Richard I. Fisher, Marc Lippman, Joan Bull, Charles Myers, Jacqueline Whang-Peng.
Vincent T. DeVita, Director, DCT, NCI
Stephen Rosenberg, Chief, Surgery Branch, COP, DCT, NCI
Harmar Brereton, Senior Investigator, Radiation Branch, COP, DCT, NCI
Eli Glatstein, Chief, Radiation Oncology, COP, DCT, NCI
Costan Berard, Head, Hematopathology Section, LP, NCI
Chemotherapy Research Nurses: Susan Hubbard, Dianne Howser, Audrey Barlock, Jane Cassidy
First Year Clinical Associates, Medicine Branch, COP, DCT, NCI

COOPERATING UNITS (if any)
Radiation Oncology Branch, NCI; Clinical Pharmacology Branch, NCI; Surgery Branch, NCI; Immunology Branch, NCI; Laboratory of Clinical Investigation, NIAID

LAB/BRANCH
Medicine Branch

SECTION
Clinical Oncology Program, National Cancer Institute, NIH, Bethesda, MD 20014

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
25	18.5	6.5

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Medicine Branch is a major clinical facility of the National Cancer Institute. Its activities are divided between clinical therapeutic trials in patients with cancer and clinically related laboratory research. Clinical trials of cancer treatment in the following diseases are currently underway: Breast Cancer, Ovarian Cancer, Hodgkin's Disease, Non-Hodgkin's Lymphomas, Melanoma, Pancreatic Carcinoma, Sarcomas, Chronic Myelogenous Leukemia. In addition Phase I-II Clinical Trials are underway, studying the following new experimental agents: Gallium Nitrate, AMSA, PALA, Chlorozotocin, Maytansine, and Cis-dichlorodiammine platinum. Additional summaries of clinical studies are summarized under reports entitled Clinical program in Breast Carcinoma and Whole Body Hyperthermia - Clinical Investigations. Laboratory research of the branch is summarized under reports entitled Clinical Pharmacology of Antineoplastic Agents, Kinetics of Cellular Proliferation, In Vitro Marrow Granulocyte Culture, Cytogenetic and Hematopoietic Stem Cell, Immunological Aspects of Cancer, Mechanisms of Hormone Dependence of Human Malignancy.

MAJOR ACCOMPLISHMENTS IN 1977:

Ovarian Carcinoma:

Established: (1) For the first time a combination chemotherapy program (Hexa-CAF) which was more effective than the previous drug of choice (L-PAM) in advanced ovarian cancer. Median duration of survival for patients treated with Hexa-CAF is 29 months, almost 1 1/2 years longer than previously published therapies for the disease.

(2) The importance of histologic grade as an independent prognostic factor in advanced disease was established for the first time.

(3) Identified and characterized a murine ovarian tumor model which is useful in defining pharmacological disposition of drugs of potential utility in ovarian cancer.

(4) Identified an increased risk of acute leukemia associated with long term alkylating agent therapy in ovarian cancer.

Published: Reviews of the status of staging and treatment of ovarian cancer; the activity of Hexamethylmelamine in alkylating agent-resistant ovarian cancer. The failure of Adriamycin as a salvage agent in progressive ovarian cancer.

Breast Carcinoma:

Established: (1) The importance of steroid hormone receptors as a prognostic factor in chemotherapy as well as endocrine treatment. Lack of estrogen receptor is associated with an Increased Response Rate to Cytotoxic Chemotherapy in metastatic breast cancer.

(2) Probability of response to endocrine manipulation in advanced breast cancer is related to the quantitative, as well as qualitative, presence of the estrogen receptor.

(3) The risks of Tamoxifen-induced retinopathy in patients receiving high doses of this anti-estrogen.

Non-Hodgkin's Lymphoma:

Established: (1) The importance of histologic conversion on the relapse frequency, response to therapy and subsequent survival of patients with non-Hodgkin's lymphoma.

(2) Important morphological differences identifiable by light microscopy will identify groups of Diffuse Histiocytic

Lymphoma patients which are now curable with established combination chemotherapies.

(3) The activity of anti-Thymocyte globulin as a specific immunotherapy against human T Cell Lymphomas.

(4) The equivalence of combination chemotherapy (CVP) with Total Body Irradiation and CVP in the management of advanced Nodular Lymphoma.

Published: Comprehensive studies on CNS complications of Lymphomas, the role of abdominal CAT scanning in Non-Hodgkin's Lymphoma, the manifestations of Lymphoma presenting in bone and GI-tract, and defined the effect of prior radiotherapy on tolerance to chemotherapy in Non-Hodgkin's Lymphoma.

Hodgkin's Disease:

Established: Important patterns of relapse after chemotherapy in advanced Hodgkin's Disease. These studies provide the basis for the use of combined modality therapy (x-ray and MOPP) in the management of the disease.

Published: Comprehensive review of the immunological aspects of Hodgkin's Disease.

Soft Tissue Sarcomas:

Established: The importance of adjuvant chemotherapy with Adriamycin-Cytoxan in improving the survival and disease-free survival of patients with adult Soft Tissue Sarcomas.

Other major accomplishments in 1977 will be found in the individual project title reports for the Medicine Branch:

Z01-CM-03420-08-M
Z01-CM-06500-04-M
Z01-CM-03404-06-M
Z01-CM-06700-04-M
Z01-CM-06702-02-M
Z01-CM-06119-08-M
Z01-CM-03423-06-M

Publications:

Ovarian Cancer:

1. Reimer, R.R., Hoover, R., Franmeni, J.F. and Young, R.C.: Acute leukemia following alkylating agent therapy of ovarian cancer. New

Engl. J. Med. 297: 177-181, 1977.

2. Fisher, R.I., Young, R.C.: Advances in the staging and treatment of ovarian cancer. Cancer 39: 967-972, 1977.

3. Young, R.C. and Anderson, T.: Ovarian and testicular malignancies. Progress in management of tumors derived from the genital ridge. Recent Advances in Cancer Treatment.: E.O.R.T.C. Monograph, Raven Press. 325-342.

4. Anderson, T. and Young, R.C.: Recent advances in the staging and treatment of ovarian cancer. Med. Clin. No. America 61: 1001-1011, 1977.

5. McGuire, W.P. and Young, R.C.: Clinical trials in ovarian cancer. Recent Advances in Cancer Treatment E.O.R.T.C. Monograph. Ed.: M. Staguet. Raven Press. (In Press).

6. Fisher, R.I. and Young, R.C.: Chemotherapy of ovarian cancer. Surg. Clin. No. America 58: 143-150, 1978.

7. Young, R.C. and Fisher, R.I.: The staging and treatment of epithelial ovarian cancer. Cancer Med. Assoc. J. (In Press).

8. Johnson, B.L., Fisher, R.I., Bender, R.A., DeVita, V.T., Chabner, B.A. and Young, R.C.: Hexamethylmelamine in alkylating-agent resistant ovarian carcinoma Cancer. (In Press).

9. Chabner, B.A., Jacobs, S., Stoller, R.G., Hande, D. and Young, R.C.: Methotrexate disposition in humans: case studies in ovarian cancer and following high-dose infusion. Drug Metabolism Rev. (In Press).

10. Young, R.C.: Current status of controlled studies in staging and therapy of ovarian cancer. Proceedings of the XVII Post-Gruaduate Course on Gynecological Tumors. Editor: H. Emanuelli. (In Press).

Breast Cancer:

11. Young, R.C., Lippman, M.E., DeVita, V.T., Bull, M., Tormey, D.: Prospectives in the treatment of breast cancer: 1976. Ann. Int. Med. 86: 784-798, 1977.

12. Chabner, B.A., Cohen, M., and Lippman, M.E.: Adjuvant therapy of breast cancer: accomplishments and future course. JAMA (In Press).

13. Lippman, M.E., Allegra, J.C., Thompson, E.B., Simon, R., Barlock, A., Green, L., Huff, K.K., Do, H.M.T., Aitken, S.C. and Warren, R.: Lack of estrogen receptor is associated with an increased response rate to cytotoxic chemotherapy in metastatic breast cancer. New Engl. J. Med. (In Press).

14. Kaiser-Kupfer, M.I. and Lippman, M.E.: Tamoxifen retinopathy. Cancer Treatment Rep. (In Press).

15. Fisher, R.I. and Lippman, M.E.: Hormonal aspects of malignant melanoma in steroid receptors and the management of cancer. ed. by E.B. Thompson and M.E. Lippman, CRC Press (In Press).

16. Lippman, M.E., Konior Yarbro, G. and Leventhal, B.G.: Clinical correlations of glucorticoid receptors in human leukemia in steroid receptors and the management of cancer. ed by E. B. Thompson and M.E. Lippman. CRC Press (In Press).

Non-Hodgkin's Lymphoma:

17. Fisher, R.I., DeVita, V.T., Johnson, B.L., Simon, R., and Young, R.C.: Prognostic factors for advanced diffuse histiocytic lymphoma following treatment with combination chemotherapy. Am. J. Med. 63: 177-182, 1977.

18. Anderson, T., Bender, R.A., Fisher, R.I., DeVita, V.T., Chabner, B.A., Berard, C.W., Norton, L., Young, R.C.: Combination chemotherapy in non-Hodgkin's lymphoma: results of long-term followup. Cancer Treat. Rep. 61: 1057-1066, 1977.

19. Young, R.C., Johnson, R.E., Canellos, G.P., Chabner, B.A., Brereton, H.D., Berard, C.W. and DeVita, V.T.: Advanced lymphocytic lymphoma: randomized comparisons of chemotherapy and radiotherapy, alone or in combination. Cancer Treat. Rep. 61: 1153-1159, 1977.

20. Chabner, B.A., Johnson, R.E., DeVita, V.T., Canellos, G.P., Hubbard, S.P., Johnson, S.K., and Young, R.C.: Sequential staging in non-Hodgkin's lymphoma. Cancer Treat. Rep. 61: 993-997, 1977.

21. DeVita, V.T., Fisher, R.I. and Young, R.C.: The treatment of diffuse histiocytic lymphoma - new opportunities for the future. Recent Advances in Cancer Treatment. E.O.R.T.C. Monograph. Ed.: M. Staquet. Raven Press. (In Press).

22. Anderson, T., Bender, R.A., Rosenoff, S.H., Brereton, H.D., Chabner, B.A., DeVita, V.T., Hubbard, S.P., Young, R.C.: Peritoneoscopy: A technique to evaluate therapeutic efficacy in non-Hodgkin's lymphoma patients. Cancer Treat. Rep. 61: 1017-1022, 1977.

23. Bender, R.A., Johnson, B.L., Norton, L. and Young, R.C.: The effect of prior radiotherapy on the tolerance and responses to chemotherapy in the non-Hodgkin's lymphomas. Am. J. of Hemat. 2: 113-122, 1977.

24. Schaner, E.G., Head, G.L., Doppman, J.L. and Young, R.C.: Computed tomography in the diagnosis, staging and management of abdominal lymphoma. J. Comp Asst. Tomography 1(2): 176-180, 1977.

25. Reimer, R.R., Chabner, B.A., Young, R.C., Reddick, R. and Johnson, R.E.: Lymphoma presenting in bone. Results of histopathology, staging and therapy. Ann. Int. Med. 87: 50-55, 1977.

26. Zwelling, L.A., Mandell, G.L. and Young, R.C.: Peritoneoscopy: an invasive procedure without bacteremia. Ann. Int. Med. 87: 454-455, 1977.

27. Hande, K.R., Fisher, R.I., DeVita, V.T., Chabner, B.A., and Young, R.C.: Diffuse histiocytic lymphoma involving the gastrointestinal tract. Cancer (In Press).

28. Chabner, B.A., Johnson, R.E., Young, R.C., Canellos, G.P., and DeVita, V.T.: Sequential nonsurgical and surgical staging of non-Hodgkin's lymphoma. Cancer (In Press).

29. Fisher, R.I., Kubota, T.T., Mandell, G.L., Broder, S. and Young, R.C.: Regression of a T-Cell lymphoma following administration of anti-thymocyte globulin. Ann. Int. Med. (In Press).

30. Lawrence, E.C., Broder, S., Jaffe, E.S., Braylan, R.C., Dobbins, W.O., Young, R.C., and Waldman, T.A.: Evolution of a lymphoma with helper T-Cell characteristics in sezary syndrome. Blood. (In Press).

31. Young, R.C., Howser, D.M., Anderson, R., Jaffe, E. and DeVita, V.T.: CNS Lymphoma: A complication of diffuse histologies. CNS Complications of Malignant Disease. Ed.: Kay and Whitehouse, MacMillan Press. (In Press).

Hodgkin's Disease:

32. Young, R.C., Anderson, T., DeVita, V.T.: The treatment of Hodgkin's disease. Current Problems in Cancer. 1: 1-29, 1977.

33. Warren, R.D., Bender, R.A., Norton, L. and Young, R.C.: The treatment of combination chemotherapy resistant Hodgkin's disease with single agent vinblastine. Amer. J. Hem. (In Press).

34. Young, R.C., Canellos, G.P., Chabner, B.A., Hubbard, S.M. and DeVita, V.T.: Patterns of relapse in advanced Hodgkin's disease treated with combination chemotherapy. Cancer (In Press).

Pancreatic Cancer:

35. Bender, R.A. and Brennen, M.F.: The role of percutaneous liver biopsy in the pretreatment evaluation of pancreatic adenocarcinoma. Am. J. Surg. 135: 207-209, 1978.

36. Dunnick, N.R., Horvath, K., Head, G.L. and Bender, R.A.: Lymphography in surgically unresectable adenocarcinoma of the exocrine pancreas. Lymphology (In Press).

37. Bender, R.A. and Brereton, H.D.: The role of peritoneoscopy in the evaluation and treatment of adenocarcinoma of the exocrine pancreas. Gastrointestinal Endoscopy (In Press).

38. Bender, R.A.: The management of pancreatic cancer in principles of cancer treatment (ed. by S.K. Carter and E. Glatstein) McGraw Hill, New York (In Press).

39. Bender, R.A., Weintraub, B.D., and Rosen, S.W.: Prospective evaluation of tumor-associated polypeptide hormones in pancreatic adenocarcinoma. Cancer (submitted for publication).

Soft Tissue Sarcomas:

40. Rosenberg, S.A., Kent, H., Costa, J., Webber, B.L, Young, R.C., et al.: Prospective randomized evaluation of the role of limb sparing surgery, radiation therapy and adjuvant chemo-immunotherapy in the treatment of adult soft tissue sarcomas. Cancer. (In Press).

Infectious Complications of Malignancy:

41. Young, R.C., Bennett, J.E. and Chu, E.W.: Organisms mimicking pneumocystis carinii. Lancet ii 1082-1083, 1976.

42. Ihde, D.C., Roberts, W.C., Marr, K.C., Brereton, H.D., McGuire, W.P., and Young, R.C.: Cardiac candidiasis in cancer patients. Cancer (In Press).

Miscellaneous Clinical Publications:

43. Blattner, W.A., Hansen, D.E., Young, R.C. and Franmein, J.F.: Malignant mesenchymoma and congenital birth defects following prenatal exposure to phenylhydration. J.A.M.A. (In Press).

44. Bender, R.A. and Young, R.C.: Effects of cancer treatment on individual and generational genetics. Sem. Onc. 5: 47-56, 1978.

45. Whang-Peng, J. and Young, R.C.: Cytogenetic studies in leukemia. Rev. of Hem. (In Press).

46. Smyth, J.F., Young, R.C. and Young, D.M.: In vivo toxicity to lymphoid tissue by 2'-deoxycoformycin. Cancer Chemo. and Pharm. (In Press).

47. Chabner, B.A., Levine, A.S., Johnson, B.L., and Young, R.C.: Initial clinical trials of maytansine, an antitumor plant alkaloid. Cancer Treat. Rep. 62: 429-433, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-03404-07-M

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

IMMUNOLOGIC ASPECTS OF CANCER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Richard I. Fisher, Senior Investigator, MB, COP, DCT, NCI

Others: Tom Anderson, Senior Investigator, MB, COP, DCT, NCI
Robert C. Young, Chief, MB, COP, DCT, NCI
Thomas T. Kubota, Clinical Associate, MB, COP, DCT, NCI
John R. Wunderlich, Senior Investigator, IB, DCBD, NCI

COOPERATING UNITS (if any)

Immunology Branch, DCBD, NCI

LAB/BRANCH
Medicine Branch

SECTION

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, N.I.H., Bethesda,MD. 20014

TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to study alteration in the immune system caused by malignancy and chemotherapy. The status of the immune system in Hodgkin's and non-Hodgkin's lymphomas is being evaluated before, during, and after chemotherapy. Immunotherapy of nodular non-Hodgkin's lymphomas with MER has been evaluated in a randomized clinical trial with in vitro monitoring of the immune system. The status of the immune system in patients with ovarian cancer is being investigated. An animal model of ovarian cancer provides opportunities for studies of immunotherapy and chemotherapy. Autologous spleen cells are being sensitized in vitro to ovarian tumor. Similar experiments in humans are being conducted with ovarian tumor cells and autologous peripheral blood lymphocytes. Studies of the immunosuppressive properties of chlorozotocin, a new non-marrow toxic nitrosourea, are underway in both humans and mice. Infection in immunosuppressed host is being evaluated. A retrospective review of 156 cases of Candidiasis detected at post mortem examination is being completed.

Publications:

1. Fisher, R.I. and Young, R.C.: Immunologic aspects of Hodgkin's disease. In Waters, H. (ed.) Handbook of Cancer and Immunology. Garland Publishing, New York. (In Press).

2. Atkinson, J.P., Fisher, R.I., Reinhardt, R., and Frank, M.M.: Reduced concentrations of the first component of complement in hypogammaglobulinemia: correction by infusion of gamma globulin. Clinical Immunol. and Immunopath. (In Press).

3. Fisher, R.I., Kubota, T.T., Mandell, G.L., Broder, S., and Young, R.C.: Regression of a T cell lymphoma following administration of anti-thymocyte globulin. Ann. Intern. Med. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-03420-09 M

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Kinetics of Cellular Proliferation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Robert C. Young, Chief, MB, COP, DCT, NCI

Others:
Bruce A. Chabner, Chief, Clinical Pharmacology Branch, COP, DCT, NCI
Charles Myers, Senior Investigator, MB, COP, DCT, NCI
William McGuire, Senior Investigator, CTEP, DCT, NCI
Stanley Shackney, Senior Investigator, CPB, COP, DCT, NCI
Robert Ozols, Clinical Associate, MB, COP, DCT, NCI
Richard I. Fisher, Senior Investigator, MB, COP, DCT, NCI
Gershon Locker, Clinical Associate, CPB, COP, DCT, NCI
James Doroshow, Clinical Associate, CPB, COP, DCT, NCI
John Smyth, Visiting Fellow, MB, COP, DCT, NCI

COOPERATING UNITS (if any)

Clinical Pharmacology Branch, NCI
Radiation Branch, NCI

LAB/BRANCH

Medicine Branch

SECTION

Cellular Kinetics Section

INSTITUTE AND LOCATION

Clinical Oncology Program, National Cancer Institute, N.I.H., Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary goal of the Cellular Kinetics Section is to develop a better understanding of the kinetic behavior of normal and tumorous tissues and to use such information to design optimal forms of chemotherapy. Toward this end both animal studies and studies in patients have been undertaken. This year alterations in DNA synthesis induced by chemotherapy in normal and tumorous tissues have been studied after 5-FU, Methotrexate, 5-Azacytidine and Adriamycin. Kinetic studies in patients with ovarian cancer after methotrexate therapy have been carried out. Flow microfluorometry studies of ascites and solid tumors from patients with breast and ovarian tumors have been sequentially analyzed after treatment. Additional studies include flow microfluorometry studies in diffuse histiocytic lymphoma as well as studies of intraperitoneal 5-FU studies in patients with ovarian cancer with malignant ascites. Cellular kinetic studies in an ovarian carcinoma murine tumor model are presently underway.

PHS-6040
(Rev. 10-76)

Publications:

1. Young, R.C., Rosenoff, S.A., Myers, C.E., Brereton, H. and Chabner, B.A.: Alterations in DNA synthesis induced by chemotherapeutic agents *in vivo*: potential applications to clinical treatment schedules. *Growth Kinetics and Biochemical Regulation of Normal and Malignant Cells.* Year Book Medical Publishers, Inc., Chicago, Ill. 787-809, 1977.

2. Silver, R.T., Young, R.C., and Holland, J.: The importance of cellular kinetics in modern cancer chemotherapy. *Am. J. Med.* (In Press).

3. Myers, C.E., McGuire, W.P., Hiss, R.H., Ifrim, I., Grotzinger, K. and Young, R.C.: Adriamycin: the roles of lipid peroxidation in cardiac toxicity and tumor response. *Science* 197: 165-167, 1977.

4. McGuire, W., Grotzinger, K., and Young, R.C.: Alterations in ^{3}H-Thymidine incorporation into DNA and ^{3}H-Uridine incorporation into RNA induced by 5-Azacytidine *in vivo*. *Biochem. Pharm.* (In Press).

5. Smyth, J.F., Young, R.C. and Young, D.M.: *In Vivo* toxicity to lymphoid tissue by 2'-deoxycoformycin. *Cancer Chemo. and Pharm.* (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-03423-07-M

PERIOD COVERED
October 1, 1977 through September 30, 1978.

TITLE OF PROJECT (80 characters or less)

IN VITRO MARROW GRANULOCYTE CULTURE

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Joan M. Bull, Senior Investigator, MB, COP, DCT, NCI

Other: Joseph O'Donnell, Investigator, ROB, COP, DCT, NCI
Peter Carmeci, Investigator, Dept. Tech. Dev., Div. of Intramural Res., NHLBI

COOPERATING UNITS (if any)

Pediatric Oncology Branch, COP, DCT, NCI

LAB/BRANCH
Medicine Branch

SECTION

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, NIH, Bethesda, Md. 20014

TOTAL MANYEARS: 7	PROFESSIONAL: 3	OTHER: 3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The laboratory of in vitro marrow culture is concerned with granulopoiesis. The laboratory has been examining the effect of 41.8°C with and without doxorubicin, or nitrosourea on human bone marrow (CFU-C), human peripheral blood (CFU-PB) and on normal murine marrow CFU-C. Also this laboratory assays and correlates data from international laboratories testing NCI-Abbott human colony stimulating factor (HGCSF).

Future projects include correlation of the combined effects of nitrosourea + hyperthermia on murine CFU-C compared to a murine mammary tumor. The laboratory will also assay bone marrow and peripheral CFU-C, as well as serum CSF for a proposed clinical trial of NCI-Abbott HGCSF in smoldering leukemia and aplastic anemia. Trials of the photometric macrotube scanner to enumerate CFU-C continue.

PHS-6040

Publications:

Schein, P.S., Bull, J.M., Doukas, D., Hoth, D.: Sensitivity of human and murine hematopoietic precursor cells to 2-[3-(2 chloroethyl)-3-nitrosoureido]-D-glucopyranose and 1-3-Bis (2-chloroethyl)-1-nitrosourea. Cancer Res. 38:257-260, 1978.

Bull, J.M., Tormey, D.C., Li, S., Carbone, P.P., Falkson, G., Blom, J., Perlin, E., and Simon, R.: A randomized comparative trial of adriamycin versus methotrexate in combination drug therapy. Cancer 41:1649, 1978.

Ladisch, S., Poplack, D.G., and Bull, J.M.: Acceleration of myeloid recovery from cyclophosphamide-induced leukopenia by pretreatment with Bacillus Calmette-Guerin. Cancer Res. 38:1049-1051, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-03424-01-M

PERIOD COVERED
October 1, 1977 through September 30, 1978.

TITLE OF PROJECT (80 characters or less)

WHOLE BODY HYPERTHERMIA - CLINICAL INVESTIGATIONS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Joan M. Bull, Senior Investigator, MB, COP, DCT, NCI

Other: David E. Lees, Anesthesiology Service, Clinical Center
William Schuette, Investigator, BEIB, Div. Res. Services
Jacqueline Whang-Peng, Senior Investigator, MB, COP, DCT, NCI
Rosalie Smith, Nursing Dept., Clinical Center

Collaborators: G. Locker, J. Doroshow, J. Gottdiener, T. Shawker, H. Gralnick,
P. Chretien, S. Broder, A. Muchmore

COOPERATING UNITS (if any)
Anesthesiology Service, CC
BEIB, DRS
Nursing Dept., CC

LAB/BRANCH
Medicine Branch

SECTION
COP, DCT, NCI

INSTITUTE AND LOCATION
Clinical Oncology Program, DCT, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The clinical whole body hyperthermia project (WBH) has examined the feasibility and safety of inducing whole body hyperthermia temperatures to 41.8°C for periods up to 4 hours as a Phase I cancer study. This study is preliminary to combining WBH with systemic chemotherapy. Fourteen patients underwent this exposure in a phase I protocol. There was no mortality associated with the procedure. WBH treatment causes a 2-fold increase in cardiac index. Toxicity included severe fatigue in all patients. Diarrhea, nausea and post procedural fever were occasionally observed. Peripheral neuropathy occured in 4/14 patients. Responses were observed in 4/14 patients.

The current and future course of the project include 2 phase I-II studies examining the feasibility and efficacy of WBH combined with (a) adriamycin or (b) methyl CCNU.

In addition to the antitumor effect, ongoing studies include evaluation of cardiac function with adriamycin, pharmacokinetics of adriamycin and hyperthermia, immunology and peripheral nerve function studies in all patients undergoing systemic heat procedures.

-6040
(Rev. 10-76)

Publications:

Lees, D.E., Schuette, W., Bull, J.M., Peng, J.W., Atkinson, R., and MacNamara, T.M.: An evaluation of liquid crystal thermometry as a screening device for deviations of core body temperature. Anesthesia, Analgesia-Current Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-CM-06119-09-M

PERIOD COVERED
October 1, 1977, to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cytogenetic and Hematopoietic Stem Cell

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Jacqueline Whang-Peng, Head, Cytogenetic Oncology Sec., MB, COP, DCT, NCI

Other Investigators: Wind-Show Cheng, MB, COP, DCT, NCI
Susan Sieber, Lab. Chem. Pharm., ET, DCT, NCI
Harmar Brereton, ROB, DCBD, NCI
Harvey Gralnick, Clin. Path. Dept., CC
Arthur Nienhuis, H., IR, NIH
Alan Andrews, DCBD, NCI
Paul Chang, BCRC
Ira Green, Lab. Immunology, NIAID
Samuel Broder, Immunology, NCI
Joan Bull, COP, DCT, NCI
William Banfield, C, LP
Ian Magrath, Pediatric Oncology, COP, DCT, NCI
Fred Li, Epidemiology Br., NCI
Turid Knutsen, MB, COP, DCT, NCI
Elaine Lee, MB, COP, DCT, NCI
Dr. Paul Bunn, C MO, VAH

COOPERATING UNITS (if any) Laboratory of Immunology, NCI; Pediatric Oncology Branch, COP, DCT, NCI; Laboratory of Chemical Pharmacology, ET, DCT, NCI; Clinical Pathology, ET, DCT, NCI; Radiation Oncology Branch, NCI; Dermatology Branch, NCI; Epidemiology Branch, NCI; BCRC, VA Hospital

LAB/BRANCH
Medicine Branch

SECTION
Cytogenetic Oncology

INSTITUTE AND LOCATION
Clinical Oncology Program, NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The areas of investigation are:

1. Cytogenetic studies of human neoplastic, hematological, and congenital diseases and of members of high-risk cancer families.

2. Observations of chromosomal aberrations and teratogenic effects of mitotic inhibitors, such as, maytansine, pala, and other Phase I drugs using in vivo and in vitro techniques.

3. Characterization of human stem cells morphologically, histochemically and by electron microscope studies as well as the elucidation of the cellular kinetics and differentiation. We are using stem cells from the peripheral blood of normal subjects and patients with refractory anemias or other myeloproliferative disorders.

4. Detection of sister chromatid exchange in patients with xeroderma pigmentosum and in cancer families.

5. Studies involving hyperthermia in treatment of various malignant diseases.

6. Cytogenetic studies of direct preparations and tissue culture cell lines established from pleural fluids and ascities from patients with Burkitt's lymphoma.

7. Cytogenetic studies of direct preparations from lymph node, bone marrow, and peripheral blood from patients with mycosis fungoides, Sezary syndrome, and reactive lymph nodes.

8. Cytogenetic studies in long-term survival patients treated for malignant lymphoma by radio- or chemotherapy.

Publications:

1. Li, F. P., McIntosh, S., Whang-Peng, J.: Double primary cancers in 2 young sibs, leukemia in another, and dextrocardia in a fourth. Cancer 39: 2633-2636, 1977.

2. Whang-Peng, J.: Cytogenetic studies in L_2C leukemia. Fed. Proc. 36: 2256-2259, 1977.

3. Barr, R. D., Whang-Peng, J., and Perry, S.: Regulation of human hemopoietic stem cell proliferation by syngeneic thymus-derived lymphocytes. Acta Haematol. 58: 74-78, 1977.

4. Rosenthal, S., Canellos, G.P., Whang-Peng, J., Gralnick, H. R.: Blast crisis of chronic granulocytic leukemia. Morphologic variants and therapeutic implications. Am. J. Med. 63: 542-547, 1977.

5. Barr, R. D., Whang-Peng, J., and Perry, S.: Preparation of mononuclear cell subpopulations from normal human peripheral blood. Biomedicine 26: 112-116, 1977.

6. Mulvihill, J. J., Gralnick, H. R., Whang-Peng, J., Leventhal, B. G.: Multiple childhood osteosarcomas in an American Indian family with erythroid macrocytosis and skeletal anomalies. Cancer 40: 3115-3122, 1977.

7. Broder, S., Poplack, D., Whang-Peng, J., Durm, M., Goldman, C., Muul, L., and Waldmann, T. A.: Characterization of a suppressor cell leukemia: evidence for the requirement of a two T-cell interaction in the development of human suppressor effector cells. N. Eng. J. Med. 298: 66-72, 1978.

8. Whang-Peng, J., Lee, E., Knutsen, T., Chang, P., and Nienhuis, A.: Cytogenetic studies in patients with myelofibrosis and myeloid metaplasia. Leukemia Res. (In Press)

9. Whang-Peng, J., and Young, R. C.: Cytogenetic studies in leukemia, in Review of Hematology, Vol II, A. A. Gordon, R. Silber, J. LoBue (Eds.) (In Press)

10. Whang-Peng, J., Lee, E. C., and Hubbell, H. R.: Karyology in Inbred and Genetically Defined Strains of Laboratory Animals. (In Press)

11. Cheng, W-S., Cheng, A., Chu, E., and Whang-Peng, J.: Atypical fluorescent body of the interphase nuclei of buccal mucosal cells and its relationship to the Y chromosome. Acta Cytol. (In Press)

12. Cheng, W-S., Tarone, R. E., Andrews, A. D., Whang-Peng, J., Robbins, J. H.: UV-induced sister chromatid exchanges in xeroderma pigmentosum and in Cockayne's syndrome lymphocyte cell lines. Cancer Research. (In Press)

13. Sieber, S. M., Whang-Peng, J., Botkin C., and Knutsen, T.: Teratogenic and cytogenetic effects of some plant-derived antitumor agents (Vincristine, colchicine, maytansine, VP-16-213, and VM-26) in mice. Teratology. (In Press)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06500-05-M

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
CLINICAL PHARMACOLOGY OF ANTINEOPLASTIC AGENTS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Tom Anderson, Senior Investigator, MB, COP, DCT, NCI
Richard A. Bender, Senior Investigator, MB, COP, DCT, NCI

Other: Leonard Zwelling, Clinical Associate, MB, COP, DCT, NCI
Donald Murinson, Research Fellow, MB, COP, DCT, NCI
Kurt Kohn, Laboratory Molecular Pharmacology, NCI
Charles Myers, Senior Investigator, MB and Clinical Pharmacology Branch, COP, DCT, NCI
Donald V. Jackson, Investigator, MB, COP, DCT, NCI

COOPERATING UNITS (if any)
Laboratory of Molecular Pharmacology, NCI
Division of Research Services, BEI, NIH

LAB/BRANCH
Medicine Branch

SECTION
Clinical Pharmacology Section

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, N.I.H., Bethesda, MD. 20014

TOTAL MANYEARS: 6	PROFESSIONAL: 6	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pharmacology projects are underway to expand the understanding of the mechanism of various antitumor agents. Based upon this work, it is hoped that new agents with improved efficacy can be developed, or that agents presently available can be applied more rationally in clinical settings. Clinical and laboratory work have centered around four classes of compounds:

(1) Nitrosoureas--The phase I clinical trial of chlorozotocin has been completed, demonstrating that the drug indeed has mild delayed bone marrow toxicity. Phase II trials to define antitumor efficacy are being initiated. In conjunction with the phase II trial, a radioactive pharmacokinetic protocol will also be activated to study the pharmacokinetics of this compound. In the laboratory, the L-glucose analog of chlorozotocin, L-chlorozotocin, has been investigated and has been demonstrated to have comparable antitumor activity, indicating that active glucose transport does not play a role in the structure activity characteristics of this compound.

(2) Platinum Coordination Compounds--Clinical work involves a broad-based phase II trial for cis-dichlorodiammine platinum as a salvage agent in a variety of tumors. This compound's efficacy is also being indirectly evaluated in several combination chemotherapy trials, including carcinoma of the testis and ovary. Collateral clinical trials involve the initiation of a metabolic study to determine renal tubular toxicity from platinum, and a study will soon be initiated to evaluate the role of scopolamine in minimizing the nausea and vomiting of this compound. In the laboratory, it has been demonstrated that the pattern of DNA and protein crosslinking produced by cis- and trans- platinum is qualitatively and quantitatively different. Using the technique of alkaline elution, it has been demonstrated that only proteinase-resistant DNA crosslinks are biologically relevant in terms of mutagenesis and/or cytotoxicity. Further work will be carried out to elucidate these differences in biological mechanisms, including the investigation of murine tumors which have been made resistant to cis-PDD.

(3) Antimetabolites--In collaboration with Dr. Charles Myers, a competitive binding assay for fluorodeoxyuridylate has been developed. This assay has been demonstrated to be applicable to measuring FdUMP in at least one murine tumor tissue. Thus in the next year the laboratory will actively investigate the role of FdUMP levels in resistant tumor tissues. Because of the marked sensitivity of this assay, attempts will be made to assess the levels of FdUMP in human malignant effusions after treatment with 5-FU. Attempts are underway to alter the assay conditions such that methylene tetra hydrofolate could also be measured to determine the effects of folate deficiency in a variety of neoplastic and non-neoplastic conditions.

(4) Antifolate and Vinca Alkaloid Pharmacology and Pharmacokinetics--Studies aimed at assessing the critical determinants for vincristine (VCR) augmentation of methotrexate (MTX) uptake have recently been completed in an L1210 mouse tumor model in vivo. Based on evaluation of four different methotrexate doses, two different vincristine doses, and four intervals of methotrexate and vincristine administration, it appears that vincristine must be administered simultaneously with methotrexate to augment in vivo transport. Moreover, in spite of this augmentation of transport it was impossible to demonstrate any interval of administration or vincristine or methotrexate dose which was capable of augmenting survival over those of methotrexate only controls. These studies prompted a more detailed analysis of methotrexate transport at "high-dose" concentrations in the human lymphoblastoid cell line and have elucidated, for the first time, a high affinity, low capacity carrier and a low affinity, high capacity carrier for methotrexate transport. These studies have been extended and expanded to now examine similar mechanisms for vincristine transport in the same human lymphoblastoid cell line. This work has clearly demonstrated a carrier-mediated, energy-dependent carrier for vinca alkaloids in human lymphoblastoid cells. Doses of drug employed in these studies are based on our earlier work on the pharmacology of tritiated vincristine in human subjects. Our human pharmacology studies have been extended to examine the biliary excretion and metabolism of vinca alkaloids in man and have more recently begun to examine the role of drug concentration on the cytoxicity

of vinca alkaloids in L1210 murine leukemia cells and CEM lymphoblastoid cells. These studies reveal a common 50% cell kill dose in the range of $2x10^{-8}$M in both cell lines. The dose response curves are very steep in this range. Further investigations are underway. These studies are aimed at the critical determinants for vincristine response and sensitivity in four strains of murine leukemia cell lines. These studies are currently examining the role of total bound vincristine, free intracellular vincristine, and the colchicine binding capacity of both the crude cell supernatant and of the aggregated fractions in an attempt to quantitate the tubulin content of these cell lines. It seems reasonable to assume that one of these several determinants has a critical role in the vinca alkaloid-induced cytotoxic effect.

Publications:

1. Javadpour, N., Doppman, J.L., Bergman, S.M., and Anderson, T.: Correlation of computerized tomography and serum tumor markers in metastatic retroperitoneal testicular tumor. *Journal of Computer Assisted Tomography*. (In Press).

2. Warren, R.D., Nichols, A.P., and Bender, R.A.: The effect of vincristine on methotrexate uptake and inhibition of DNA synthesis by human lymphoblastoid cells. *Cancer Res* 37: 2993-2997, 1977.

3. Bender, R.A., Castle, M.C., Margileth, D.A., and Oliverio, V.T.: The pharmacokinetics of [^{3}H] - vincristine in man. *Clin. Pharmacol. and* Therap. 22: 430-438, 1977.

4. Warren, R.D., Nichols, A.P., and Bender, R.A.: The membrane transport of methotrexate in human lymphoblastoid cells. *Cancer Res.* 38: 668-671, 1978.

5. Bender, R.A. and Makula, D.R.: The methotrexate - dihydrofolate reductase interaction and its effect on DNA and tetrahydrofolate synthesis in L1210 cells *in vitro*. *Brit. J. Cancer* 37: 403-410, 1978.

6. Cohen, M., Bender, R.A., Donehower, R., et al.: Reversibiltity of high affinity binding of methotrexate in L1210 murine leukemia cells. *Cancer Res.* (In Press).

7. Jackson, D.V., Castle, M.C. and Bender, R.A.: Biliary Excretion of [^{3}H] vincristine in man: a case report. *Clin. Pharmacol. Therap.* (In Press).

8. Bender, R.A., Nichols, A.P., Norton, L. and Simon, R.M.: Lack of Therapeutic synergism between vincristine and methotrexate *in vivo*. *Cancer Treat. Rep.* (In Press).

9. Zwelling, L.A., Kohn, K.W., Ross, W.E., Ewig, R.A.G. and Anderson, T.:

The kinetics of formation and disappearance of a DNA cross-linking effect in mouse leukemia L1210 cells treated with *cis*- and *trans*- platinum (II) diamminedichloride: *Cancer Res.* (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06700-05-M

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Clinical Program in Breast Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Marc E. Lippman, Head, MBCS, MB, COP, DCT, NCI

Other Investigators:
Senior Investigators, MB, COP, DCT, NCI
First and Second Year Clinical Associates, MB, COP, DCT, NCI
Chemotherapy Nurses, COP, DCT, NCI
Jane Cassidy, Nurse, MB, COP, DCT, NCI
R. Simon, Head, Biostatistical Info. Sys. Unit, COP, DCT, NCI
Barbara Curtis, Private Practice

COOPERATING UNITS (if any)

See Above

LAB/BRANCH

Medicine Branch

SECTION

Medical Breast Cancer Section

INSTITUTE AND LOCATION

Clinical Oncology Program, National Cancer Institute, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3-1/2	2-1/2	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Medical Breast Cancer Section is responsible for the development of a clinical and laboratory program directed at breast cancer. Clinical trials of adjuvant chemotherapy and chemoimmunotherapy, and trials in metastatic disease comparing chemotherapeutic, hormonal and chemohormonal regimens are underway. Biochemical and hormonal marker studies are undertaken and coordinated by the Medical Breast Cancer Section.

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(Rev. 10-76)

Project Description:

The Medical Breast Cancer Service was established in July 1972 and the clinical program was initiated in January 1973. It was responsible to the Office of the Associate Director, COP, until its shift to the Medicine Branch in August 1974.

I. Clinical Trials

A. Adjuvant Chemotherapy ± immunotherapy.

1. B135 - This protocol comparing L-PAM to CMF to CMF plus C-parvum was closed due to inadequate accrual. A total of 55 patients entered on study will continue to be followed for DFI, survival and biological marker and immunologic factors.

B. Recurrent disease trials.

1. MB160, a randomized trial of chemotherapy ± hormonal therapy aimed at inducing cell synchrony was initiated in August of 1976. This trial has 38 patients on study and is too early for evaluation.

2. MB151, a randomized trial of hormonal therapy in first recurrent breast cancer in which steroid hormone receptors are to be evaluated prospectively. Only 16 patients have been entered thus far, and this study is too early for evaluation.

3. MB132, a randomized trial of tamoxifen ± halotestin. This trial, nearing completion (94 patients entered), has suggested preliminarily that doses of tamoxifen >12 mg/m^2 are inferior to lower doses, and that tamoxifen plus halotestin is superior in response rate.

4. A Phase II trial of CIS platinum in breast cancer is underway. Thus far there have been no significant PR's or CR's in this trial.

II. Ancillary Studies

A. Steroid Binding Proteins (SBP)

SBP are being prospectively evaluated in all breast cancer samples. These include analyses for androgen, estrogen, glucorticoid and progestin receptors. In addition, analyses are being performed on melanoma, ovary, colon, male breast and hematologic malignancies. Numerous publications resulting from these data are listed below.

III. Extramural Activities

A. Breast Cancer Task Force.

Dr. Lippman is on the Steering Committee of the Breast Cancer Task Force.

B. National Surgical Adjuvant Breast Project.

Dr. Lippman is on the Endocrine Committee of the National Surgical Adjuvant Breast Project.

Publications:

1. Lippman, M.E., Halterman, R., Leventhal, B.G., Perry, S., and Thompson, E.B.: Glucocorticoid Binding Proteins in Human Leukemia Blast Cells. Nature New Biology, 242: 157-158, 1973.

2. Thompson, E.B., and Lippman, M.E.: Mechanism of Action of Glucocorticoids. Metabolism, Clinical and Experimental 23: 159-201, 1974.

3. Gurpide, E. and Lippman, M.E.: Steroid Hormone Receptors in Breast and Endometrial Carcinoma in Current Concepts. A Scope Publication. The Upjohn Corporation, Kalamazoo, Michigan.

4. Chabner, B.A., Cohen, M., and Lippman, M.E.: Adjuvant Therapy of Breast Cancer: Accomplishments and Future Course. (In Press).

5. Alford, J.C., My Do, H., Geelhoed, G.W., Tsangaris, N.T. and Lippman, M.E.: Steroid Hormone Receptors in Human Colon Cancers. Cancer (In Press).

6. Lippman, M.E., Allegra, J.C., Thompson, E.B., Simon, R., Barlock, A., Green., L., Huff, K.K., Do, H.M.T., Aitken, S.C. and Warren, R.: Lack of Estrogen Receptor is Associated with an Increased Response Rate to Cytotoxic Chemotherapy in Metastatic Breast Cancer. (In Press).

7. Kaiser-Kupfer, M.I. and Lippman, M.E.: Tamoxifen Retinopathy. Cancer Treatment Reports (In Press).

8. Fisher, R.I. and Lippman, M.E.: Hormonal Aspects of Malignant Melanoma in Steroid Receptors and the Management of Cancer. ed by E.B. Thompson and M.E. Lippman. CRC Press (In Press).

9. Lippman, M.E., Konior Yarbro, G. and Leventhal, B.G.: Clinical Correlations of Glucorticoid Receptors in Human Leukemia in Steroid Receptors and the Management of Cancer ed by E.B. Thompson and M.E. Lippman. CRC Press (In Press).

10. Allegra, J.C., Lippman, M.E., Thompson, E.B. and Simon, R.: The Influence of Steroid Hormone Receptors on Response to Chemotherapy in Patients with Metastatic Breast Cancer in Steroid Receptors and the Management of Cancer ed by E.B. Thompson and M.E. Lippman. CRC Press (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06702-03 M

PERIOD COVERED
October 1, 1977, to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Mechanisms of Hormone Dependence of Human Malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Marc Lippman, Sr. Investigator, MB, COP, DCT, NCI
Marie E. Monaco, NIH Postdoc. Fellow, MB, COP, DCT, NCI
Fred Hendler, Cancer Expert, MB, COP, DCT, NCI
Joseph C. Allegra, Clinical Associate, MB, COP, NCI
Jeanine Strobl, George Washington Univ., Dept. of Pharmacology

Others: Susan Aitken, Georgetown Univ. Med. School
Karen Huff, Lab Tech, MB, NCI
E. Brad Thompson, Lab. Biochem. NCI
Charles Eil, NICHD
Lynn Loriaux, Endocrinology Br., NICHD

COOPERATING UNITS (if any)

Lab. Biochem., NCI and Endocrinology Branch, NICHD

LAB/BRANCH
Medicine Branch

SECTION
Medical Breast Cancer Section

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, Bethesda, Maryland 20014

TOTAL MANYEARS: 10	PROFESSIONAL: 10	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating the mechanisms whereby steroid and polypeptide hormones stimulate growth and specific protein synthesis in human breast cancer both in tissue culture model systems we have established and in clinical settings.

1. We are studying the prevalency and clinical correlates of specific steroid receptors for estrogen, androgen, glucocorticoid and progesterone in human breast cancer, lymphomatous diseases, melanoma, colon carcinoma, ovarian cancer and male breast cancer.

2. Assays for specific gene products (α-lactalbumin, casein, fatty acid synthetase, thymidine kinase) have been developed and we are studying the effects of steroid hormones, insulin and prolactin on the activities and synthesis of these proteins.

3. We are investigating glucocorticoid receptors in various subpopulations of normal and leukemic lymphoid cells, including their regulation by mitogens

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(Rev. 10-76)

such as phytohemaglutinin. In addition we are studying glucocorticoid receptors in Burkitts Lymphoma, hairy cells leukemia, ANLL and lymphomas.

4. We have developed a vasopressin responsive rat mammary cancer cell line in tissue culture. This is the first *in vitro* long term vasopressin dependent system to be characterized and as such, appears particularly promising for the study of the mechanism of vasopressin action.

5. We are characterizing an activity derived from bovine mammary gland which appears to be inhibitory of but not lethal to breast epithlial cells in culture and as such may qualify as a putative chalone.

6. We have developed a completely defined hormone supplemented medium (T3 Insulin transferrin and estradiol) which supports the growth of human mammary cancer in long term tissue culture.

Publications

1. Lippman, M. and Barr, R.: Glucocorticoid Receptors in Purified Subpopulations of Human Peripheral Blood Lymphocytes. *J. Immunol* 118: 1977-2003, 1977.

2. Yarbro, G.S., Lippman, M.E., Johnson, G.E. and Leventhal, B.G.: Glucocorticoid Receptors in Subpopulations of Childhood Acute Lymphocytic Leukemia. *Cancer Research* 37: 2688-2695, 1977.

3. Monaco, M.E., Osborne, C.K. and Lippman, M.E.: Insulin Stimulates of Fatty Acid Synthesis in Human Breast Cancer Cells. *JNCI* 58: 1591-1593, 1977.

4. Lippman, M.E., Huff, K., Bolan, G. and Neifeld, J.P.: Interactions of R5020 with Progesterone and Glucocorticoid Receptors in Human Breast Cancer and Peripheral Blood Lymphocytes *in vitro* (ed. by W.L. McGuire et al.) Raven Press, NY, 1977.

5. Thompson, E.B., Norman, M. and Lippman, M.E.: Steroid Hormone Actions in Tissue Culture Cells and Cell Hybrids. Their relation to human Malignancies. Recent Progress in Hormone Research (In Press)

6. Osborne, C.K., Monaco, M.E., Lippman, M.E. and Kahn, C.R.: Insulin Receptors in Human Breast Cancer Cells in Long Term Tissue Culture: Correlation between insulin binding, degradation and biologic activity. *Cancer Research* 38: 94-102, 1978.

7. Monaco, M.E., Lippman,M.E. and Kidwell, W.R.: Prolactin Responsive Rat Mammary Tumor in Long Term Tissue Culture. *PNAS* (In Press)

8. Lippman, M.E.: Effects of Glucocorticoids on Human Lymphoid and Leukemia Cells *in* Mechanisms of Glucocorticoid Action. (ed. by Rousseau, G. and Baxter, J.D.) Spring-Verlag (In Press)

9. Osborne, C.K. and Lippman, M.E.: Human Breast Cancer in Tissue Culture The Effects of Hormones in Experimental Breast Cancer (ed. by McGuire, W.L., Plenum Press (In Press)

10. Monaco, M.E. and Lippman, M.E.: Hormone Action in Human Breast Cancer Cell Lines in Endocrine Control of Neoplasia (ed. by Criss, W.E.) Raven Press.

11. D'Agata, R., Monaco, M.E., Loriaux, D.L. and Lippman, M.E.: Lack of Aromatization of Androstenedione by Human Breast Cancer in Tissue Culture. Steroids (In Press)

October 1, 1977 to September 30, 1978

ANNUAL REPORT OF THE NCI-VA MEDICAL ONCOLOGY BRANCH OF THE DIVISION OF CANCER TREATMENT, NATIONAL CANCER INSTITUTE AND THE WASHINGTON VETERANS ADMINISTRATION HOSPITAL

1.0 Summary

1.1 Description

The NCI-VA Medical Oncology Program is an intramural branch of the DCT/NCI/NIH and a section of the Medical Service, and a section of the Research and Education (R & E) Service of the Washington, D. C. Veterans Administration Hospital (WVAH) and thus designated as a Program by the WVAH and VACO. The primary goals of the NCI-VA M.O.B. are: investigation of patients with malignant disease in clinical therapeutic trials and laboratory investigations of tumor cell biology. Secondary aims are to provide a service function to the WVAH for management of patients with malignant disease on a consultation basis.

1.2 Administration

The NCI-VA M.O.B. is a cooperative program between the COP/DCT/NCI/NIH and the VA with a contract signed by the Director NCI and the VA central Office (VACO) providing for the transfer of funds from the DCT to VACO and the provision of ward, office, outpatient clinic and research laboratory space and billets by the VA to the NCI. Both Veteran and non-Veteran patients are entered into clinical protocols approved by clinical review committees of the WVAH and the NCI.A letter of agreement has been prepared to define the guidelines under which the unit operates; part of the agreement will be signed by the directors of NCI and Medicine (VACO); the other parts will be signed by the Chief, NCI-VA Oncology Program and the ACOS for R and E, WVAH. Drafts of these letters have been submitted to the VA for concurrence.

1.3 Clinical Resources

All clinical and ward supporting work for protocol and non-protocol patients is performed by 5 senior staff oncologists, 1 infectious disease specialist, 2 pathologists, 6 first year and 3 second and third year clinical associates, 2 rotating interns, 2 pharmacists, 3 research nurses, 22 full time and 4 part time nurses, 1 medical technologist, 3 ward and outpatient clinic secretaries, 1 medical DMT;from WVAH funding part time dietitian, food service worker, social worker, housekeeping aid, and supply technician are supplied. The inpatient unit has 30 beds and an outpatient treatment area. The outpatient clinic runs 3 half days a week in the WVAH outpatient area, and 5 days a week in the inpatient ward area. A satellite ambulatory care program (SACP) with 5 rooms runs at the United Inn, Bethesda, Md. as part of the COP/DCT outpatient facility contract.

1.4 Clinical Patient Load

The NCI-VA M.O.B. assumes complete primary care including inpatient and outpatient responsibility for all patients on its protocols and in addition, primary care for Veteran patients deemed likely to have significant benefit from complex chemotherapy treatment regimens by the NCI-VA Chief (@ 300 patients). The program also provides consultative services for all patients with malignant disease as requested in the inpatient and outpatient areas, and provides outpatient chemotherapy and care for WVAH veterans requiring non-protocol chemotherapy (approximately 100 patients in FY 78).

The program sees 30 to 40 new patients a month in consultation. The 2CN beds have been over 90% occupied, and there are approximately 240 outpatient visits per month. Non-veteran inpatient occupancy has been approximately 30% of the total, and non-veteran outpatient visits approximately 20-25% of the total. A number of the veteran patients come for protocol treatment from outside the WVAH area. All transportation costs for protocol patients are met by the NCI transferred funds.

The senior staff provides 12 months a year of inpatient attending coverage on the general medical service, 2 months a year attending coverage on the infectious disease consultation service, supervises the WVAH tumor registry, participates in the WVAH cancer committee and weekly Medical-Surgical Chest conference, provides a weekly consult rounds conference and multiple individual conferences for the WVAH through the general hospital education program. The pathologists provide additional opinions on histologic material to the hospital.

1.5 Oncology Fellowship Training Program

Under the auspices of the COP/DCT/NCI the NCI-VA M.O.B. provides for clinical training in the subspecialty of Medical Oncology for the Clinical Associates assigned to it primarily (as special positions designated by the VACO) or to the USPHS officers rotating from the clinical center, NIH. In addition, it provides general medical and oncology training to twelve interns from the Medical Service, WVAH, George Washington University and Georgetown University that rotate through the inpatient wards.

1.6 Laboratory Research Facilities and Staffing

The laboratory research program in tumor cell biology is carried on in 300 square feet of wet lab space, and the program has the use of common research space including instrument, storage and animal rooms totally an additional 1500-2000 square feet of space. There are 7 senior investigators, 1 post doctoral fellow (PhD), 4 clinical Associates, and 11 technicians working in this area.

1.7 Major New Developments

Dr. William West has joined the senior staff as an Oncologist and has established an immunology research lab at the WVAH. The plan is to form a laison with the Laboratory of Immunodiagnosis, DCBD (Dr. Herberman, Chief).

Dr. Eli Glatstein has recently come as the new head of the Radiation Oncology Branch DCT and we have spent considerable time developing new protocols with him as collaborative ventures. The patients for these protocols will be irradiated and chemotherapy in limited stage small cell lung cancer; hemibody radiotherapy in lung cancer and myeloma; plans for electron bean radiotherapy of mycosis fungoides (which presently is being done at Howard University).

With Dr. Reed Dunnick we have established collaborative ventures in evaluating computer assisted tomography of NCI-VA M.O.B. patients at the CC/NIH.

For both of the above we have proceeded to contract a van to transport patients between the NCI-VA Unit and the CC/NIH.

1.8 Summary of Clinical Investigations.

Diseases being studied by the NCI-VA Medical Oncology Branch include: bronchogenic carcinoma, especially small cell bronchogenic carcinoma, prostate carcinoma, gastric carcinoma, hepatocellular carcinoma, T-cell lymphomas including mycosis fungoides and the Sezary syndrome, and multiple myeloma.

The major treatment study involves small cell bronchogenic carcinoma. Chemotherapy programs tested in this disease include a randomized trial of cyclic alternating combination chemotherapy with two (CMC, VAP) or three (CMC, VAP, VP-16-IF) non-cross resistant drug combinations without radiation therapy (Protocol 75-4). Significant treatment advances have been recorded in small cell carcinoma. In our most recent trial the predicted and observed 28 month disease-free survival was 13% for all patients and 23% for patients demonstrating a complete tumor response (CR) by clinical staging. We found the CR rate was increased by addition of the second combination, but no increase was seen with the third combination. These results are probably better than those of other small cell carcinoma trials reported in the literature that employed chemotherapy alone or with radiation therapy. Especially gratifying was the fact that 10% of patients with disseminated disease (extensive stage) were long duration survivors. These patients comprise an extremely poor prognostic group in most small cell carcinoma trials.

Thymosin fraction V from calf thymus was tested in a prospective randomized trial (Protocol 75-4) in collaboration with the S.B./DCT in small cell carcinoma. We found thymosin 60 mg/M2 treated patients survived significantly longer than patients in the untreated group or receiving 20 mg/M2 thymosin. Survival prolongation resulted from prolongation of the duration of the initial complete response. Analysis of other factors has not revealed an explanation for these differences and we are continuing to study thymosin immunotherapy in a randomized fashion in our small cell carcinoma trials. (Protocols 77-1, 77-3, 77-4)

In protocol 77-1, we tested the addition of chest radiotherapy after standard CMC VAP induction chemotherapy and found no additional complete responders and as yet no differences in survival. We are also analyzing these patients for chest relapse.

Our present ongoing studies in small cell carcinoma are designed to extend and/or confirm the above results. We have systematically evaluated the sites of relapse in SCBC and found a high incidence of chest relapse in limited stage patients given chemotherapy along. In protocol 77-4 we are conducting a randomized trial in collaboration with the ROB/DCT of combination chemotherapy plus or minus simultaneous chest radiotherapy during induction to ask whether the radiotherapy will help control intrathoracic disease. We have demonstrated that the combined modality therapy is well tolerated, and the trial is ongoing.

In extensive disease (ED) small cell carcinoma we have tested a new induction regimen (CAPO in protocol 77-3. This regimen is intensive, and complete response (CR) rate for ED stage patients will probably exceed the 40% CR rate attained with alternating CMC VAP chemotherapy. Neuropathy has been encountered as a problem and adjustments in the regimen may have to be made. The trial is ongoing.

Other therapeutic trials currently being performed or ended in small cell carcinoma include a published phase II trial of streptozotocin (protocol 76-5), phase II trial of high dose methotrexate (HDMTX) with leucovorin rescue (LVFR),a phase II trial of Cis-platinum-diamine-dichloride, phase II trial of cytosine arabinoside, of hemibody radiotherapy (in collaboration with ROB/DCT).

We have studied the results of chest radiotherapy given to palliate relapses of SCBC from chemotherapy alone and found that extremely poor local tumor control occurs in this situation.

We are developing a protocol for autologous bone marrow storage and transplantation following ablative chemotherapy and total body irradiation for extensive stage small cell lung cancer in collaboration with the Experimental Hematology Section of the POB/DCT.

A series of studies have been conducted on the natural history and staging of lung cancer, particularly SCBC. The growth rates of small cell carcinoma have been measured from tumor volumes on serial pretreatment X-rays and the median doubling time found (77 days) is much longer than previously reported in the literature and has important implications with regard to design of chemotherapy regimens.

Central nervous system involvement by small cell carcinoma was studied in a clinical and autopsy review of 204 patients. Tremendously high (over 80% at 2 years) actuarial probability of developing CNS metastases was found. In addition, the propensity for epidural, leptomeningeal involvement and multiple sites of involvement along with the failure of nitrosoureas to prevent CNS metastases were found. This data is important for planning future trials of CNS prophylaxis.

Several histopathologic studies relating to small cell carcinoma were performed. We found no statistically significant effect of histologic subtype of SCBC on clinical presentation, response rate, response duration or survival. Morphologic transitions of small cell carcinoma to other cell types were also found in autopsy tissues. The presence of epidermoid, adenocarcinoma, or large cell anaplastic carcinoma elements was noted in over 50% of evaluated cases. Cases with morphologic transformations had decreased survival relative to patients with SCBC without these transformations. Thus, this appears to be an important histopathologic correlation of this disease.

The effects of bone marrow involvement by small cell carcinoma were evaluated. Marrow disease responded to chemotherapy, and patients tolerated intensive chemotherapy well. No significant differences were found in hematologic parameters in patients with and without marrow involvement.

Infectious complications such as herpes zoster noted in 12% of patients with SCBC 7 - 18 months after intensive treatment, and a 10% incidence of interstitial lung disease were found in the SCBC patients.

In collaboration with CTEP/DCT we have been developing a world wide registry of long term survivors of SCBC to be used for study. Acute leukemia was noted in a long term survivor of SCBC.

A large effort had been devoted to finding drugs and drug combinations with anti-tumor activity, against non-small cell carcinoma (adenocarcinoma, large cell anaplastic carcinoma, epidermoid carcinoma, and mixed histology large cell small cell carcinoma). The major problem in this group of lung cancers still involves finding treatment programs that will produce significant numbers of complete responders among patients with inoperable disease. At present several regimens, including the bleomycin, methotrexate and streptozotocin regimen developed at our branch produce 30-40% partial response rates.

In protocol 76-9, 28 non-small cell lung cancer patients were treated with high dose methotrexate by 6 or 30 hour infusion and citrovorum factor rescue We found the regimen could be given safely to older patients with lung cancer, and there was no renal, cardiac, liver or bone marrow toxicity. However, only 2 objective responses were seen. There was no difference in the 6 or 30 hour regimen. and we conclude non-small cell lung cancer is resistant to this treatment.

In protocol 76-10 ifosfamide and ascorbic acid have been tested and no activity against non-small cell lung cancer seen.

We have identified a subset of large cell lung cancer with components of small cell lung cancer (mixed histology lung cancer) and have treated these with intensive combination chemotherapy protocols designed for small cell cancer. We have found a 90% objective tumor response rate, but a low complete response rate, and low median survival (6 months) in this group.

We have been developing protocols for non-small cell lung cancer that include identifying the 30-40% chemotherapy responders and then debulking the intrathoracic disease in these patients and continuing chemotherapy With this we are planning atrial of hyperalimentation to study the effects of this modality on chemotherapy response and toxicity. In addition, we are planning trials of autologous peripheral blood leukocyte reconstitution after cumulative myelosuppression by chemotherapy (in collaboration with the EHS/POB), and a trial of active specific immunotherapy with autologous tumor cells taken from the resected patients (in collaboration with LID/DCBC).

In gastric carcinoma, we have complete a trial employing alternating cycles of 5-FU, methyl CCNU and adriamycin-mitomycin C. (Protocol 76-8) 18 patients were treated but partial response was noted only in 11%, and disease stabilization in 61% by applying strict response parameters. Median survivals were comparable to other literature reports. We conclude this tumor is not as sensitive to chemotherapy as previously reported. We found gastric fluid CEA was a good indicator of tumor presence, but gastroscopy was not useful in following response to therapy. A phase II trial of ICRF 159 (Protocol 76-17) is ongoing.

In advanced, stage D prostate cancer failing hormonal manipulation a protocol (76-6) evaluating combination chemotherapy with cyclophosphamide and adriamycin has been completed. In 20 patients the objective response rate was 35%, and the median survival of responders will exceed 12 months. The value of bone scans, plasma CEA, lymphangiograms, skeletal X-rays, serum acid phosphatase, and performance status were evaluated as parameters of response to therapy. Also, a few patients were seen to have bone fide objective second responses to hormonal manipulation.

A new protocol (77-8) has been designed to test the role of combination chemotherapy (cyclophosphamide, adriamycin, cis-platinum) in stage D prostatic carcinoma patients who have had no prior hormonal manipulation. Collaborators include (Dr. Javadpour) the SB/DCT and (Dr. Lippman) MB/DCT who will assist in prostatic biopsies and assays for hormone receptors. After failing chemotherapy the patients will receive full hormonal manipulation and the response will be evaluated. In addition, diagnostic procedures include lymphangiography and pelvic CAT scans with Diagnostic Radiology CC/NIH.

Protocols for combination chemotherapy and combined modality electron beam radiotherapy in cutaneous T-cell lymphomas (Mycosis Fungoides-Sezary Syndrome) MF-S Protocol 76-12) are ongoing in collaboration with Howard University Radiotherapy, and ROB/DCT. 20 patients with Staff III through V MF-S have been treated with vinblastine, adriamycin and bleomycin (VAB) and electron beam radiotherapy, and later alternating cycles of VAB and cyclophosphamide

Methotrexate and prednisone. Response rates and duration of advance disease patients are greater in the program than have been reported for treatment with either electron beam therapy or chemotherapy alone.

For Stage I, II MF-S disease, electron beam radiotherapy followed by low dose IV nitrogen mustard therapy for systemic disease is being given. 10 patients have been treated.

Studies of the natural history and biology of T-Cell lymphomas include: cytogenetic studies (with Dr. Peng MB/DCT) which reveal abnormal cytogenetics in most patients. Morphologic characteristics of neoplastic T-cells as demonstrated by electron microscopy (by Dr. J. Guccion Laboratory Service, WVAH); and immunologic studies indicate that malignant T-cells may retain functional characteristics. (In collaboration with Drs. Broder, Waldman, Metabolism Branch/DCBD; Dr. Schechter, Hematology, WVAH).

Other studies in Mycosis Fungoides Sezary Syndrome include: staging studies of bipedal lymphangiography, peritoneoscopy, and phase II trials of high dose methotrexate with citrovorum factor rescue; phase II trials of VP-16-213, phase II trial of antithymocyte globulin.

In multiple myeloma and other plasma cell malignancies we are evaluating cyclic alternating combination chemotherapy employing melphalan, prednisone, with vincristine, cyclophosphamide, adriamycin, prednisone. (Protocol 76-7). 21 patients have been treated and the response rates are similar to those expected with melphalan prednisone alone although relapsing patients on melphalan, prednisone demonstrate response on the second combiantion. Phase II trials in myeloma patients include VP-16-213 and hemibody radiation therapy.

In non-resectable hepatocellular carcinoma patients we are evaluating the combination of adriamycin and streptozotocin. (Protocol 76-11). Prognostic factors influencing treatment response and survival have been identified. These include the serum bilirubin level and the patient's performance status. Responses are only noted in good prognosis patients. One patient who was non-resectable prior to chemotherapy became clinically resectable while on treatment. Serum alpha fetoprotein determinations have been a relatively accurate reflection of total tumor mass.

Phase I trials ongoing or in development include: testing of dichloromethotrexate (Protocol 78-1); PALA (in collaboration with Dr. Chabner, LCP/DCT, Protocol 78-3): pentamethylemelamine (in development).

We have been systematically studying staging procedures in the evaluation of the natural history and management of malignant disease with particular emphasis on evaluation of response to treatment. Fiberoptic bronchoscopy (FOB) was found to be a very useful tool for following lung cancer patients and their response to treatment. In addition, using FOB early tumor progression can be detected, as well as providing material for histologic, cytologic and cell biologic studies.

Other staging procedures include peritoneoscopy (performed by members of the branch) in Mycosis Fungoides-Sezary syndrome, gastric cancer; gastroscopy for gastric cancer (performed by GI-Hepatology Section, WVAH), and computer assisted tomography (CAT Scans) performed by Dr. R. Dunnick, CC/NIH in prostate cancer, small cell carcinoma, hepatocellular carcinoma and gastric ancer.

We are evaluating tumor markers in the natural history and response to treatment. These include a lung cancer associated antigen (in collaboration with Dr. McIntire, LID/DCBD); paraneoplastic syndromes with Dr. Hawley, Neurology Service, WVAH; calcitonin, with Drs. Becker, and Silva, Endocrinology Section, WVAH.

Other clinico-pathologic correlations being made by our pathologists Dr. Matthews and Dr. Gazdar include: correlation of clinical response with subtypes of small cell carcinoma of the lung; correlation of tumor bulk, relapse sites, and cause of death in combined modality versus chemotherapy in small cell carcinoma; development of different lung cancer histology after treatment with chemotherapy and chemotherapy-radiotherapy; evaluation of gastric cytologies in gastric carcinoma; histopathologic correlation of gastric carcinoma in surgically resected patients; evaluation of bone marrow biopsies in small cell carcinoma,, correlation of histologic subtyping with survival in surgically resected lung cancer; reference pathology for lung cancer for the Eastern Cooperative Oncology Group; a prospective study of histologic criteria for diagnosis of Mycosis Fungoides -Sezary syndrome.

Infectious disease studies conducted by Dr. Fossieck (in collaboration with Infectious Disease Section, WVAH) included monitoring of infection during episodes of severe pancytopenia in patients with solid tumors undergoing intensive combination chemotherapy;(we have found that fever is common but documented infection is rare ; evaluation of gram positive coccal bacteremia in patients with neoplastic disease; bacteremia in patients with Mycosis Fungoides-Sezary syndrome; development of herpes zoster in patients with solid tumors; development of Pneumocystis carinii and other opportunistic infections in patients with solid tumors; bacteremia during staging procedures such as bronchoscopy and peritoneoscopy; evaluation of the bacteriology of the lower respiratory tract in patients with lung cancer; clinicopathologic correlation of candida infections in cancer patients (in collaboration with Dr. Brereton ROB/DCT): and interstitial lung disease in patients with solid tumors receiving intensive combination chemotherapy.

A prospective study of hepatitis virus transmission in an oncology unit and at the time of fiberoptic bronchoscopy has been started in collaboration with Drs. Seef and Hoofnagel of the Hepatology Section, WVAH.

In collaboration with the Cardiology Section (Drs. Katz, DiBianco and Fletcher, WVAH) we have evaluated the usefulness of hemodynamic measurements and conservative management of cardiac tamponade developing as a result of lung cancer in 14 patients and found these methods excellent.

We have developed computer programs to store and retrieve information on data on patients in clinical trials.

Several literature of malignant disease have been prepared and published.

The nursing staff have been active in educational pursuits on both a national and local level, with particular emphasis on lung cancer, and Mycosis Fungoides.

The research pharmacy lab under Mr. Pelsor (in collaboration with the Clinical Pharmacology Branch/DCT) has been developing specific methods for the analysis of biological fluid concentrations of antineoplastic drugs. An assay for methotrexate is in use, and this will be employed to also monitor dichloromethotrexate protocols. An assay for adriamycin and its metabolites is being developed. Also drug model studies on VP-16-213 have been performed.

1.9

SUMMARY OF LABORATORY STUDIES

In the laboratory, we are attempting to answer some of the questions regarding fundamental tumor cell biology, with a view to developing newer methods to treat human cancer. Areas of investigation include cell biology studies *in vitro* and *in vivo* (as heterotransplants), special nutritional requirements of tumor cells, cell kinetics, genetics of malignant cells and oncogenic viruses, tumor antigens and the immunological responses to malignancy, hybrid cells that produce high titer monospecific antibodies, chemotherapy and combined modality regimens, and the early laboratory diagnosis of the infectious complications of malignancy.

New models for the study of lung cancer, especially small cell carcinoma (SCCL) have been established *in vitro* and as heterotransplants, and have been well characterized. They retain many of the properties of the original tumors, but advantages and disadvantages of each model system have been identified. Attempts to establish new models for cutaneous T cell lymphomas have been met with only limited success.

SCCL is a rapidly growing tumor in the patient, but grows relatively slowly *in vitro*. Its growth appears dependent on special growth factors released by other cells. Identification of such factors could lead to new methods to control its growth in the patient.

Over 100 human tumors have been implanted into athymic nude mice, with varying degrees of success. Successful heterotransplantation is dependent on several factors, including histological type, organ of origin, endocrine secretion or dependence, and whether the tumor is primary or metastatic. Contamination with xenotropic stain of murine leukemia virus (MuLV) occurs frequently. Metastases are rare. Subcutaneous tumors grow as slowly expanding masses, and do not directly lead to death. Intracranial inoculation is more sensitive, and leads to invasive lesions that rapidly induce death.

Cell hybridization techniques are used extensively for a variety of reasons. They have been used to identify the chromosomal locations of the genes coding for many isozymes of man, mouse, hamster and rabbit. These studies have also lead to the identification of regions of homology between the chromosomes of man and other species, and provide important insights into evolutionary patterns. Interspecies hybrid cells have also been used to try and identify genes associated with malignancy, and also genes capable of suppressing the malignant phenotype. Conventional methods of growing hormone secreting cells have had only limited success, and we are attempting to grow insulin producing cells in long term culture by fusing them to a variety of different cell types.

A rapidly evolving field, of tremendous theoretical and practical significance, is the use of B x B lymphocyte hybrid cells (hybridomas) for the production of high titer, monospecific antibodies. Improved methods for their establishment, cloning, maintenance and antibody characterization have been developed. Initial attempts, in collaboration with several immunology laboratories, have concentrated on producing antibodies against antigens that have been previously well defined. Attempts are being made to identify new tumor antigens by these techniques, and also to make antibodies against polypeptides secreted by tumors.

A major effort has been made to identify cellular genes required for type C RNA tumor viruses, using hybrid cells formed by fusing permissive with non-permissive parental cells. We have previously identified mouse chromosome 5 as being necessary for replication of ecotropic strains of MuLV, and chromosome 8 (or 19) for replication of amphotropic strains. Using radiolabelled gp71 (the major envelope glycoprotein) of an ecotropic strain, we have demonstrated that gp71 binding and replication segregate together in hybrid cells, and we have localized the gene coding for the virus specific cell surface receptor to mouse chromosome 5. We have studied the genetic requirements for spontaneous expression of MuLV by comparing activation in spleen cells and hamster x mouse spleen hybrid cells. Mouse chromosome 7 is required for ecotropic virus expression in AKR mice, and apparently is necessary but not sufficient in BALB/c mice.

Using hybrid cells the human chromosomal requirements for replication of primate tropic type C viruses have also been investigated. Baboon M7 virus requires human chromosome 19. The chromosomal requirements for replication of wooley monkey virus SSAV differ from those for M7 virus. While they have not been completely identified, the possibility exists that more than one chromosome may supply the required functions for SSAV. Attempts to map the human integration sites of the primate tropic viruses using rodent x preinfected human cell hybrids have been complicated by lack of complete resistance to infection by some of the rodent parental lines, and the possibility that interspecies transfer of the virogene may occur. However, the isolation of a small number of virus free subclones may eventually lead to the identification of the human integration sites.

Dr. Cohen's Laboratory has investigated blood flow through tumors. Impaired tumor blood flow may affect the response to chemotherapy. He has found evidence of considerable stress within the tumor vascular bed, and is attempting to investigate agents that may improve the flow.

Dr. Fossieck's Laboratory has continued its work on the development and evaluation of counterimmunoelectrophoresis (CIE) assays for the early detection of infection. Along with tests for S. pneumoniae, K pneumoniae, H. influenzae, N. meningiditis, S. aureus and C. albicans, tests for M. pneumoniae and A. fumigatus antibodies have been instituted. An animal model for the study of endocarditis has been perfected and is in use. In a study involving simulated and clinical blood cultures, it was shown that CIE could identify organisms which are adaptable to the method as soon as or shortly after the gram stain becomes positive. This is at least 24 hours sooner than the subculture method.

Dr. Bunn's Laboratory has investigated the kinetics of human malignancies. The doubling times of SCCL has been found to be much longer than previously thought. The majority of cells from multiple myeloma and Sezary's syndrome are aneuploid. He and Dr. Shackney have found that the malignant cells in the skin of Sezary's patients do not originate there but migrate from elsewhere. This finding should have major implications in the proper management and treatment of this disease. Dr. Bunn has also investigated the DNA content of megakaryocytes, and is measuring shifts in ploidy after stimulation with a variety of agents.

Dr. Ihde has investigated the effects of tumor oxygenation on radiation and chemotherapy sensitivity. Under different conditions, hyperbaric oxygenation will increase or decrease the labelling index of an experimental tumor. The toxicity of cyclophosphamide is enhanced by hyberbaric oxygen exposures that reduce the labelling index, but not under conditions that increase it.

Dr. West, a recent addition to the senior staff, has established an immunology laboratory. He has begun studying both animal models and clinical material from patients on treatment trials. Using an experimental animal lung cancer model, he has demonstrated a weak proliferative response of lymphocytes to tumor-associated antigens, susceptibility to natural killer (NK) cells *in vitro*, and striking susceptibility of this tumor to activated macrophages. In studies of autologous tumor cells and lymphocytes from SCCL patients, he has found proliferative responses of lymphocytes to autologous human tumor cells. In addition, SCCL appears highly susceptible to NK cells. However, patients with SCCL appear to have decreased total NK activity. Exposure to interferon inducers *in vitro*, appears to increase the NK activity. Our plan is to use Dr. West to form a bridge with the Laboratory of Immunodiagnosis DCBD (Dr. Herberman, Chief), and to use these immunologic studies to monitor the current clinical trials and aid in designing new immunotherapy trials in patients.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03024-09 VAMO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Clinical Trials and Miscellaneous Clinical Investigations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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William H. West, M.D.	LIB/DCBCD &	NCI-VA MOB NCI/WVAH (USPHS)

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(Continued)

COOPERATING UNITS (if any)

LAB/BRANCH
NCI-VA Medical Oncology Branch

SECTION
None

INSTITUTE AND LOCATION
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TOTAL MANYEARS: 60	PROFESSIONAL: 17	OTHER: 43

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study new methods of evaluating and treating malignant diseases. Therapeutic approaches include: combination chemotherapy and combined modality chemotherapy with radiotherapy and immunotherapy. Intensive induction regimens, alternating combinations, low dose combination regimens, new agents (high dose methotrexate with citrovorum rescue, streptozotocin, ICRF 159, Ifosphamide, thymosin, hemibody radiotherapy) are tested. The major cancers studied include: lung (small cell, epidermoid, adenocarcinoma and large cell carcinoma); hepatocellular; gastric; prostatic; multiple myeloma; and mycosis fungoides-Sezary syndrome. New methods of evaluating response to therapy are explored with: fiberoptic bronchoscopy, peritoneoscopy, gastroscopy, cat scans & tumor cell markers. Complications of therapy are studied such as: fever, infection, interstitial pulmonary disease, metabolic and nutritional derangements. Clinical histopathologic correlations are made before and afer therapy. Computer programs for storage, retrieval and manipulation of clinical research information are developed.

PHS-6040
(Rev. 10-76)

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(Continued)

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T. Shields, M.D.	Chairman VASAG Lung Committee	
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2.0 CLINICAL TREATMENT STUDIES

2.1 Small Cell Carcinoma of the Lung (SCBC)

2.101 Randomized trial of alternating cyclic combination chemotherapy (CMC VAP + VPIF) (Dr. Cohen and Senior Staff, Protocol 75-4).

Sixty-one patients with small cell bronchogenic carcinoma (SCBC) were treated with cyclic alternating combination chemotherapy. No chest or prophylactic brain radiation therapy was employed. 28 months after starting treatment, a patient's projected and observed free survival probability was 23% for patients achieving a complete response (CR) and 12% for all patients. All patients were initially treated with a combination of cyclophosphamide, methotrexate and CCNU (CMC) given intensively for 6 weeks. Irrespective of response to CMC they received vincristine, adriamycin, and procarbazine (VAP) for the next 6 weeks. The addition of VAP increased the complete response rate from 42% to 74% for limited stage patients and from 24% to 36% for extensive stage patients. At 12 weeks the patients were randomized to receive a third drug combination containing VP-16-213 and ifosfamide (VPIF) for 6 weeks. Patients randomizing to VPIF received cyclic alternating chemotherapy at 6 week intervals with CMC, VAP and VPIF while the remaining patients received alternate cycles of CMC and VAP. Addition of this third combination did not further increase the complete response rate or prolong survival. Therapy was continued in complete responders for 2 years.
We conclude in SCBC that: 1.) long term disease free survival can be achieved with intensive combination chemotherapy alone in a large fraction of patients; 2.) addition of a second combination of non-cross reactive drugs increases the CR rate and hence the survival duration but a third combination added no further response or survival benefit; 3.) from analysis of relapse data we find that responses achieved earlier were associated with increased relapse free survival; 4.) development of complete response documented by careful restaging (see section 2.9) is associated with prolonged survival; 5.) analysis of prognostic features revealed that performance status, sites of disease and attainment of a complete response are better predictors of survival than conventional staging systems.

2.102 Combination Chemotherapy followed by chest radiotherapy in SCBC. (CMC, VAP + Chest RT) (Dr. Cohen and Senior Staff, Protocol 77-1).

Following the study mentioned in 2.101, we tested the addition of chest radiotherapy following CMC, VAP induction chemotherapy. Patients were then maintained on alternating cycles of CMC VAP. Thus these patients are otherwise comparable to the patients in Protocol 75-4 that receive CMC VAP. Patients received 2000 rads in 1 week. 15 patients were entered, and objective and complete response rates and survival were not different from the previous study of chemotherapy alone. Specifically, no additional patients went into a complete response after the addition of chest radiotherapy. The analysis of relapse in the chest has not been completed.

We conclude from these studies that while the addition of chest radiotherapy after combination chemotherapy can be achieved with relatively little additional toxicity there is no significant benefit when compared to patients who received chemotherapy alone in the previous trial, Protocol 77-4).

2.103 Randomized Trial of Combination Chemotherapy ± simultaneous chest radiotherapy during induction (combined modality therapy) for limited stage SCBC. (CMC ± Chest RT, VAP). (Dr. Cohen and Senior Staff in collaboration with Dr. Glatstein, ROB/DCT.

Following the two studies mentioned in 2.101, 2.102, and the information generated by a previous ROB SCBC study which showed a large percentage of relapse free long term survivors in SCBC treated with induction chemotherapy and simultaneous radiotherapy we have designed a trial to ask whether the addition of simultaneous radiation therapy starting on day 1 of induction chemotherapy will increase the complete response rate, the early CR rate, and the duration of long term disease free survival. Patients with limited stage SCBC are randomized to CMC VAP chemotherapy alone (as in 75-4) or to combined CMC VAP chemotherapy and radiation therapy to the primary chest lesion (in collaboration with the ROB/DCT). Patients receive 4000 rads in 3 weeks. 12 patients have been entered, and it is as yet too early to tell if there are differences between the two arms. There have been no severe complications and no treatment related deaths in either arm. This is very important because the prior ROB combined modality trial had a large number of severe treatment related complications and deaths.

2.104 Intensive Combination Chemotherapy of extensive stage SCBC. (cyclophosphamide, adriamycin, VP-16-213, Vincristine, CAPO) (Dr. Minna and Senior Staff, Protocol 77-3).

The prior work of our branch, the ROB, and other reported studies have shown that patients with extensive stage SCBC, particularly with extrathoracic disease while responding initially to therapy have a significantly lower CR rate, shorter median survival and 10 fold less long term (2 year) disease free survivors than patients with limited stage disease. In addition, randomized trials suggest that local radiotherapy at present does not help extensive stage disease in terms of survival. We have sought to explore new induction regimens for patients with this disease stage. We have combined 4 highly active agents and given them in an intensive fashion in the CAPO regimen, (all drugs are given on days 1,8,22,29,43,50 in full doses). Maintenance is with moderated doses for outpatient management. 9 patients have been entered. There has been 1 treatment related to death. Four of the 9 patients are probably complete responders. The hematologic toxicity has been comparable to the prior regimens, but there has been neuropathy of moderately severe degree seen in 3 patients that is reversible by switching the patients to CMC maintenance. We plan to continue this protocol with some modifications for another 5-10 patients. It is to early to determine survival and none of the patients has yet relapsed.

2.105 Randomized trial of Thymosin Immunotherapy in SCBC. (Dr. Cohen and Senior Staff, in collaboration with Drs. Chretien SB/DCT, and Dr. Makuch, Statistical Branch, DCT, Protocols 75-4, 77-1, 77-4, 77-3).

Thymosin fraction V (prepared by Hoffman-LaRoche Inc., Mr. J. Synder) from calfs has been shown to reconstitute children with hereditable immunodeficiency disorders of the thymus. Because of known deficits in cell mediated immunity in lung cancer, and those that probably occur with intensive combination chemotherapy, we have been testing in randomized trials the use of the agent in SCBC. Patients were randomized to receive thymosin 60mg/M2, 20 mg/M2, or no thymosin treatment during their first 6 weeks of induction chemotherapy. Thymosin treated patients received the drug twice weekly for 12 doses. To date the effects of thymosin in protocol 75-4 have been fully analyzed. The 3 treatment groups were comparable with regard to prognostic factors affecting treatment response and survival. There were no differences in the complete response rate to chemotherapy in the 3 groups. Thymosin 60mg/M2 treated patients survived significantly longer than patients in the other two treatment groups. Survival prolongation resulted from prolongation of the duration of the initial complete response. Following disease relapse there were no survival differences in the 3 treatment groups. Because of this result we have dropped the 20mg/M2 arm. Because of the undefined nature of thymosin (a mixture of polypetides), and the potential importance of this result we are continuing to randomize patients to receive thymosin 60 mg/M2 or no thymosin in current SCBC trials which then will be analyzed. The mechanisms by which thymosin increased survival duration is not yet known but may relate to restoration of immune deficits due to disease or treatment. To aid in this, Dr. West of our senior staff has established laboratory methods for investigating the potential effects of thymosin. (See Laboratory Section).

2.106 Phase II Trial of Streptozotocin for SCBC (Dr. Bunn and Senior Staff, Protocol 76-5).

A phase II trial of this non-myelosuppressive agent has been published. 13 patients with SCBC who had failed primary combination chemotherapy were treated with streptozotocin. No response was seen. We conclude that this agent, despite its lack of myelotoxicity has little or no activity in SCBC.

2.107 Phase II Trials of High Dose Methotrexate (HDMTX) with Leucovorin Rescue (LVFR). (Dr. Minna and Senior Staff, Mr. Pelsor, Protocol 76-9).

In searching for other non-myelosuppressive regimens for SCBC, and in an attempt to see if patients failing standard dose MTX would respond to HDMTX with LVFR a phase II trial of this regimen is being conducted. 12 patients who have failed other combination chemotherapy on the primary protocols have received HDMTX with LVFR by 6 hour bolus, or 30 hour continuous infusion. No responses have been seen. Some bone marrow toxicity has been seen in these heavily treated patients who have received extensive prior chemotherapy. So far only 5 patients have received more than 1 course. We are continuing this study until 14 patients have been evaluated .

2.108 Phase II Trial of Cis-Platinum in SCBC and other tumors (Dr. Cohen, Huberman, Thant and Senior Staff, Protocol 77- 7).

Patients with SCBC and other tumors who have failed primary treatment protocols and high dose methotrexate (see 2.107) are being treated with cis-platinum. No responses have been seen yet in the few patients treated.

2.109 Phase I-II trial of hemibody radiotherapy in SCBC (Dr. Ihde and Senior Staff in collaboration with Dr. Glatstein R.O.B./DCT, Protocol 77-2).

SCBC is highly responsive to radiotherapy initially. We are investigating the antitumor effect of this modality given as systemic treatment (hemibody radiotherapy) in collaboration with Dr. Eli Glatstein, Chief, Radiation Oncology Branch, DCT, NCI. Patients failing primary therapy receive 200 rads/week in fractionated schedules and tumor responses are evaluated. If responses are seen, the plan is to combine this relatively non-myelosuppressive treatment with chemotherapy in a combined modality fashion in freshly-diagnosed patients during induction therapy. So far 5 patients have been treated and no responses have been seen. Thrombocytopenia has occurred in these patients previously heavily treated with chemotherapy.

2.110 Evaluation of radiotherapy in control of local disease in SCBC after relapse on combination chemotherapy. (Dr. Ihde and Senior Staff).

We have reviewed the records of 23 patients who developed progressive small cell lung cancer on combination chemotherapy without prior radiotherapy who then received local radiotherapy in an effort to palliate their local disease.

Objective response rate was only 53% to radiotherapy and median time to local disease progression was only 3 months. The median radiation course was 3300 rads in 11 fractions. Concomitant administration of new chemotherapy and higher radiation doses had a suggestive effect on increasing the local response rate. We conclude that palliative doses of radiation therapy after failure of chemotherapy in this tumor gives extremely poor local tumor control in a disease thought to be relatively sensitive to radiotherapy.

2.11 Phase II Trial of Cytosine Arabinoside (ARA-C) in SCBC Responders at the Time of Maximal Response. (Dr. Shackney and Senior Staff, Protocol 75-4).

Four patients with SCBC undergoing primary treatment (2 complete responders and 2 partial responders) were given several courses of cytosine arabinoside (ARA-C) at the time of maximal tumor response to see if this cycle specific agent would have a large tumor effect as predicted from cell kinetic arguments. However, we found that tumor regrowth began quickly after the Ara-C and there was no hint of further tumor shrinkage. We conclude from this small trial that ARA-C is inactive in SCBC even in a potentially favorable situation and this report is being prepared for publication.

2.112 Autologous Bone Marrow Storage and Transplantation Following Ablative Chemotherapy and Total Body Irradiation in Extensive Stage Small Cell Lung Cancer. (Dr. Minna and Senior Staff, in collaboration with Drs. Abrams and Deisseroth, POB/DCT, and Dr. Glatstein, R.O.B/DCT, Protocol in Development)

We have spent considerable time planning a protocol of ablative chemoradiotherapy in extensive stage small cell lung cancer. The plan is: to administer several cycles of induction chemotherapy over a 6-8 week period as per our previous experience; then give outpatient chemotherapy, and a brief rest period so that by week 12 bone marrow will have recovered. At this time autologous bone marrow will be harvested under general anesthesia and stored under vapour phase N2 by the P.O.B/DCT Experimental Hematology Section, a group experienced in this procedure. The patient will then be given high dose, and potentially marrow ablative chemotherapy, followed by total body irradiation 600-800 rads and then marrow re-infusion. Extensive studies of bone marrow function *in vitro* as well as standard clinical parameters of bone marrow function will be conducted before and after transplantation. The complete responders will receive prophylactic cranial irradiation, but otherwise receive no maintenance chemotherapy. Several questions will be answered by this approach: 1) can durable, long term disease free unmaintained survivors be generated? 2) can partial responses to standard induction chemotherapy be converted to complete responses by the intensive consolidation regimen? 3) will relapses be in bone marrow before other sites? 4) since the patients are unmaintained after this consolidation treatment, what is the time or regrowth of clinically viable detectable tumor off therapy?

Because of the toxicity associated with continued maintenance combination chemotherapy, even with tumor regrowth the overall quality of life may be better for these patients in the unmaintained state; 5) will it be possible to give chemotherapy to relapsing patients after autologous marrow engraftmant?

2.113 Development of CNS Metastases in Small Cell Carcinoma. (Dr. Bunn, Dr. Matthews, and Dr. Nugent).

The clinical and autopsy records of 204 patients treated by the NCI-VA MOB for SCBC without prophylactic cranial irradiation (PCI) were reviewed for evidence of CNS metastases. Thirteen percent of patients presented with CNS involvement at the time of initial treatment, and at death, metastases were found in over 50% of patients. The cumulative probability of developing CNS metastases increased with increasing survival such that over 80% of patients are predicted to have CNS metastases by 2 years after starting treatment. Other important findings include: 1) the occurence of multiple sites of involvement; 2) the frequent occurence of epidural and leptomeningeal involvement sites outside of the radiation ports treated with PCI; 3) residual tumor in sites treated with chemo and irradiation therapy at autopsy indicating the lack of effectiveness in treating bulk disease in the CNS; 4) pituitary and cerebellar metastases are present more frequently than previously appreciated; 5) the lack of effect of introsourea chemotherapy in preventing the development of CNS metastases. We conclude from these studies, that in order to obtain long term disease free survivors in SCBC prophylactic therapy of the entire neuraxis will have to be undertaken.

2.114 Tumor Doubling Times in SCBC (Dr. Shackney, Bunn, Brigham)

The radiographs of 144 patients with SCBC were reviewed and 12 were found suitable for accurate serial doubling time measurements. Tumor doubling times ranged from 25 to 160 days with a mean of 91 days. A major conclusion is that the long doubling times in small cell carcinoma may be associated with a long subclinical latent period between response and tumor recurrence even when log kill is small. Our current feeling is that cure should not be entertained in patients with disease free intervals less than 4-5 years. Because of the selection of radiographs (representing) only 10% of the total initial group) it is possible that selection factors for subsets of SCBC may have occurred.

2.115 Development of acute leukemia in long term survivors of SCBC. (Dr. Minna, Dr. Bradley and Dr. Schechter, Hematology, WVAH).

A long term (over 2 years) disease free survivor with SCBC treated with combination chemotherapy has developed progressive pancytopenia with particularly severe thrombocytopenia, shoulder arthritis, with positive bone scans and a bone marrow and peripheral blood picture consistent with acute erythroleukemia. We conclude that SCBC long term survivors receiving chemotherapy are at risk of developing leukemia as reported for other tumors, and this case is being prepared for publication.

2.2 Adenocarcinoma, Large Cell Anaplastic Carcinoma, Epidermoid Carcinoma, and Mixed Histology (Large Cell: Small Cell) Carcinoma of the Lung.

The major problem in this class of lung cancers is to find chemotherapy regimens that produce objective and complete responses in a large percentage of patients.

2.21 High Dose Methotrexate by Six Hour Infusion and Citrovorum Factor Rescue (HDMTX). (Dr. Minna and Senior Staff, Protocol 76-9)

Twenty-eight patients with unresectable non-small cell lung cancer have been treated with HDMTX and CVFR by either 6 hour infusion in doses up to 12 grams/M2, or 30 hour infusion in doses of 1.5 gm/M2. Plasma MTX levels were monitored by a competitive binding radioassay. Peak MTX levels were 10^{-2}M in the 6 hour infusion, and 5×10^{-5}M in the 30 hour infusion. Patients were extensively staged including fiberoptic bronchoscopy; all patients had measurable or evaluable disease and 88% had received no prior chemo or radiotherapy. There have been 2 objective responses, no hematologic or renal toxicity and the median survival for all patients was 6.5 months. There was no significant survival difference for patients treated on the 6 or 30 hour regimens. We conclude that HDMTX with CVFR can be given safely to adult patients with lung cancer, however, the tumor response rate is very low.

2.22 Ifosfamide and Ascorbic Acid. (Dr. Cohen and Senior Staff Protocol 76-10).

A few patients have been entered on this protocol and no objective responses have been seen.

2/23 Combination Chemotherapy of Mixed Histology Large Cell: Small Cell Lung Cancer (WHO 22/40). (Dr. Minna, Dr. Matthews and Senior Staff, Protocols 75-4, 77-4).

We have identified 19 patients with a mixture of large cell and small cell carcinoma in their histologic specimens obtained during diagnostic evaluation. These patients would have been scored as large cell anaplastic carcinoma by most pathologists.

These patients have been treated on intensive combination chemotherapy protocols (CMC; CMC, VAP; CMC, VAP+RT) designed for SCBC. Three complete responses, and 14 partial responses have been seen for an overall objective response rate of over 90%. The response rate for specific sites were: endobronchial disease 64%, Pulmonary parenchymal disease 73%, lymph node 67%, and liver 100%. However, the duration of most of these responses is short and the median survival for the entire series is only 5 months. The 6 longest survivors lived 10.2, 11.8, 14.7, 21.1, 24.3, and 26.8 months. We conclude, that mixed histology large cell/small cell lung cancer (WHO 40:22) is a distinct clinical entity that: may often be scored as large cell carcinoma and hence may represent many of the responses for this histologic type of lung cancer reported in the literature; have a low complete response rate compared to SCBC but a high objective tumor response rate in all tumor sites studied when given intensive combination chemotherapy; and that while the therapy has yet made little overall impact on patient survival a subset of patients do appear to have survival benefit. Thus, for both treatment and investigational purposes the entity must be distinguished from SCBC and the other lung cancer histologic types.

2.24 Combination Chemotherapy Plus Surgical Resection in Stage III, Non-Small Cell Lung Cancer. (Dr. West and Senior Staff, in collaboration with the Surgical Service, Drs. Schafer and Higgins, WVAH; Protocol in Development).

At least eight of ten patients with non-small cell lung cancer have systemic disease at the time of diagnosis. The presence of microscopic tumor in mediastinal nodes is a clear indicator of systemic dissemination, even in patients whose tumor appears otherwise limited to the lung. These patients would be logical candidates for systemic chemotherapy in addition to surgical removal of clinically evident intrathoracic disease. Unfortunately, cytotoxic drugs are only marginally effective in this disease. A variety of combination chemotherapy regimens reported over the past two years have yielded objective tumor response rates of 30-40%, but the complete response rate has remained low.

We plan to select patients with non-small cell lung cancer confined to the chest but inoperable on the basis of a positive mediastinoscopy and treat them with a six week course of intensive chemotherapy. Those patients demonstrating objective tumor shrinkage from chemotherapy will then undergo surgical resection of intrathoracic disease. Postoperatively these patients will continue adjuvant chemotherapy for six months. This will permit a Phase II analysis of the ability of patients to tolerate surgery after intensive combination chemotherapy and will alsoexamine the potential benefit of preselecting patients for surgery and adjuvant drug therapy on the basis of their response to chemotherapy.

2.25 Randomized Trial of Hyperalimentation in Non-Small Cell Lung Cancer. (Dr. West and Senior Staff). (Protocol in Development).

Since the combination of intensive chemotherapy and thoracotomy imposes major additional nutritional and hematologic demand on patients already compromised by malignant disease, this study will also investigate the potential benefit of aggressive nutritional and hematologic support in this patient population. During the initial chemotherapy trial patients will be randomized to receive hyperalimentation through a catheter in the superior vena cava vs. routine diet. Several studies have suggested but have not irrefutably demonstrated that HA can increase the proportions of responders among patients receiving intensive chemotherapy. It has been convincingly demonstrated that HA can ameliorate hematologic and gastrointestinal toxicity. We will examine these issues in a randomized, prospective fashion.

2.26 Trial of Autologous Peripheral Blood Leukocytes to Ameliorate Cumulative Myelosuppression (Dr. West and Senior Staff, Protocol in Development).

In collaboration with the Experimental Hematology Section of the Pediatric Oncology Branch (Dr. Diesseroth and Dr. Abrams) of the NCI we will also investigate the capacity of peripheral blood mononuclear cell preparations (5 x 10^9 cells per an IBM cell separator) to ameliorate hematologic toxicity during the adjuvant phase of chemotherapy. It is anticipated that cumulative myelosuppression will result in progressively severe nadirs or more frequent delays in treatment in latter months of adjuvant treatment. Buffy coats will be harvested and cryopreserved prior to adjuvant chemotherapy and will be tested for their effect on bone marrow recovery in patients manifesting progressive hematologic intolerance to drugs.

2.27 Randomized Trial of Active Specific Immunotherapy (Dr. West and Senior Staff, in collaboration with Dr. Herberman LID/DCBC, Protocol in Development).

Finally, the availability of surgically-resected autologous tumor material from all patients lends itself to a trial of active specific immunotherapy in the post-operative period with irradiated autologous tumor cells (10^7) plus BCG (10^8) in a common injection site on two occasions seven days apart as demonstrated effective in the guinea pig hepatoma model. Patients selected for surgery will be randomized to receive this therapy vs. hematologic support. Those randomized to immunotherapy will also undergo buffy coat harvesting (ten days after BCG-tumor cell challenge) but will receive buffy coat re-infusion only after all chemotherapy has been completed. This will permit analysis of the potential use of these buffy coats in "immunologic re-constitution", as monitored by serial assays of the lymphoproliferative and lymphokine response to autologous tumor membrane as well as by assays of delayed hypersensitivity, lymphocyte count, E-rosette count, and natural cytotoxicity.

2.3 Gastric Carcinoma

2.31 Alternating cyclic combination chemotherapy of gastric cancer. (5-flurouracil, Methyl CCNU; adriamycin, mitomycin-C, FM,AM)(Dr. Bunn and Senior Staff, Protocol 76-8).

18 previously untreated patients with unresectable gastric cancer were treated with alternating two drug combinations (FM,AM) employing four active single agents. Extensive serial staging procedures were employed including GI series, gastroscopy with gastric washings, bone and liver scans, bone marrow biopsy, and direct inspection of the liver at surgery or peritoneoscopy. At the completion of one complete cycle of the four drugs only 2 patients (11%) had achieved a partial response by rigidly defined objective parameters, disease stabilization occurred in 11 patients (61%), and progressive disease in 5 patients (28%). No additional PR's were noted after this time. Median survival for the 3 groups were 38+, 30 and 14 weeks respectively and 27 weeks for the entire group. The PR+ stable disease rate is comparable to PR rates reported in other series as is the median survival, and survival curve for the series as a whole. We conclude that despite intensive combination chemotherapy, by using strict response criteria that gastric carcinoma is a less sensitive tumor to chemotherapy than previously reported. (see also 2.93)

2.32 Phase II Trial of ICRF 159 in Gastric Carcinoma (Dr. Bunn and Senior Staff, Protocol 76-17).

ICRF has been reported to be active against several gastrointestinal tumors. 5 patients with gastric cancer have been entered in a phase II trial and one response has been seen to date.

2.4 Carcinoma of the Prostate

2.41 Combination Chemotherapy (Cyclophosphamide plus Adriamycin) of hormone resistant prostatic carcinoma with secondary hormone manipulation. (Dr. Ihde and Senior Staff, Protocol 76-6).

The efficacy of combination chemotherapy in hormone-resistant metastatic carcinoma of the prostate and evaluation of various methods of assessing tumor response and progression are being investigated. Patients are treated with cyclophosphamide and adriamycin, two agents with demonstrated activity in this disease, and after tumor progression are given a hormonal therapy to which they have not previously been exposed. Objective responses, either shrinkage of tumor masses or normalization of an elevated serum acid phosphatase level or both, have been observed in 7 of 20 patients (35%). Median survivals will exceed 12, 7 and 4 months respectively in patients with partial response, stable disease for 4 months, and progressive disease, suggesting that therapy may be having an impact on survival. Severe hematologic toxicity was seen in 5 patients; 3 had previously received radiotherapy, and patients who had been given this modality are no longer being accepted for combination chemotherapy.

One of 8 patients has objectively responded to subsequent hormone manipulation despite previous hormone resistance, suggesting that a minority of patients may retain hormone responsiveness after an initial progression. Bone scan, plasma CEA, and node size on lymphangiogram improved in half or more of the responders; bone scan, CEA, serum acid phosphatase, skeletal X-rays, body weight, and performance status worsened in half or more cases of objective tumor progression. Initial pain relief was equally common in patient who did not eventually respond to chemotherapy.

2.42 Combination Chemotherapy (Cyclophosphamide, Adriamycin and Platinum) followed by hormonal therapy for previously untreated metastatic carcinoma of the prostate with hormone therapy (orchiectomy) at progression, and sequential studies of hormone receptors on prostate carcinoma cells. (Dr. Ihde and Senior Staff in collaboration with Drs. Lippman (MB) and Dr. Javadpour (S.O.B.), Protocol 77-8).

A new protocol designed to test the role of combination chemotherapy in patients with metastatic tumor who have not had estrogens or orchiectomy has recently been approved. Patients initially will have tumor biopsied for hormone receptor proteins, and then will receive cyclophosphamide, adriamycin and cis-platinum with evaluation of response. This will be followed by orchiectomy at disease progression with evaluation of the tumor response at this stage. Hormone receptor proteins will be monitored prior to each phase of treatment.

2.5 Cutaneous T Cell Lymphomas: Mycosis Fungoides-Sezary Syndrome (MF-S)

2.51 Combination Chemotherapy (Vinblastine, adriamycin, bleomycin, (VAB) alternating with cyclophosphamide, methotrexate and prednisone (CMP) and Electron Beam Radiotherapy for Stage III, IV and V MF-S (Dr. Bunn and Senior Staff in collaboration with Dr. Schechter Hematology, WVAH, Dr. Fischman Dermatology, WVAH, Dr. Kumar, Radiotherapy Howard Univ. and Dr. Glatstein, R.O.B./DCT, Protocol 76-12)

20 patients have been entered on this protocol. Patients are treated with combined modality therapy including electron beam irradiation and combination chemotherapy. Electron beam irradiation alone in these stages produces high response rates but the duration of response is rarely, if ever, longer than 1 year. Similarly, single agent chemotherapy produces responses in 60-100% of patients but these responses are also of short duration (less than 1 year). The combination chemotherapy in this trial was chosen on the following principles: 1.) each agent is active when used alone; 2.) bleomycin and adriamycin have radiosensitizing properties; 3.) combinations of adriamycin, bleomycin and vinblastine are active in other lymphomas; 4.) low dose continuous chemotherapy may be more appropriate than high dose intermittent therapy in the treatment of slowly proliferating malignant lymphomas.

2.52 Combined Modality Electron Beam Radiotherapy Followed by Low Dose Systemic Nitrogen Mustard (HN2) Chemotherapy for Stage I and II MF-S. (Dr. Bunn and Senior Staff in collaboration with Drs. Schechter, Fischman, Kumar and Glatstein - Protocol 76-12)

In earlier stage MF-S (Stage I and II) electron beam radiotherapy has been shown to cure 30% and 10% of patients respectively. Other centers have combined electron beam therapy with topical HN2. Systemic low dose HN2 is the most active single agent in MF-S and has little bone marrow toxicity. Thus we have combined electron beam radiotherapy (2400 rads in 6 weeks) followed by 3, 2-week cycles of HN2 (2mg/day) in an attempt to treat skin disease, microscopic areas of visceral disease and/or disease outside the radiotherapy depth of penetration. Ten patients have been entered, all have responded, and toxicity has been acceptable.

2.53 Phase II Trial of High Dose Methotrexate with Citrovorum Factor Rescue (HDMTX, CVFR) in Cutaneous T Cell Lymphomas failing other Chemotherapy. (Dr. Minna and Senior Staff - Protocol 76-9)

HDMTX with CVFR has been used in other lymphomas with success and it would be useful to have a non-myelosuppressive regimen for this disease. Thus, we are treating patients with MF-S that have failed primary therapy (including regimens with standard dose MTX) with HDMTX and CVFR. No objective responses have been seen to date.

2.54 Phase II Trial of VP-16-213 in Cutaneous T Cell Lymphomas Failing other Chemotherapy. (Dr. Bunn and Senior Staff - Protocol 76-12)

VP-16-213 has been anecdotally reported to be active against MF-S, and it is known to have activity against other lymphomas. Thus, we are systematically studying its activity in patients failing primary chemotherapy. So far one response has been seen.

2.55 Phase II Trial of Anti-thymocyte Globulin (ATG) in Cutaneous T-Cell Lymphomas failing Other Chemotherapy. (Dr. Bunn and Senior Staff in collaboration with Dr. Fisher, M.B./DCT and Dr. Broder, L.M./DCBD -Protocol 77-6)

Horse anti-thymocyte globulin has been used in human trials of organ transplantation and has been shown to kill T lymphocytes . We have administered this agent to three patients and seen 1 dramatic response, with clearing of skin lesions and normalization of an extremely high peripheral blood lymphocyte count (115,000 to 8,000). Serum sickness has been a major problem. Because ATG is a unique and potentially specific model for passive humoral immunotherapy of human tumors, we are continuing work with this protocol.

2.56 Cytogenetic Studies of Mycosis Fungoides-Sezary Syndrome. (In collaboration with Dr. J. W. Peng, M.D./DCT)

The cytogenetics of MF-S have not yet been worked out. We have systematically been obtaining peripheral blood, bone marrow and lymph nodes for cytogenetic analysis. The majority of patients have cells with abnormal cytogenetics and chromosome banding studies should reveal whether this is restricted to certain chromosomes.

2.57 Functional Properties of T Cells from Patients with Mycosis Fungoides Sezary Syndrome. (In collaboration with Dr. Broder and Waldman, Metabolism Branch/DCBD, and Dr. Schechter, Hematology, WVAH).

The T Cell properties of malignant cells from MF-S patients are being studied. Dr. Broder and Dr. Waldman have found that Sezary cells may have the ability to "help" or "suppress" antibody production by lymphocytes in vitro. In addition, an interesting patient with disappearing bones is being studied to see if his cells produce osteoclast activating factor by Dr. Schechter. These studies represent an effort to understand the clonal nature and biology of the malignant MF-S cells.

2.58 Ultrastructural Analysis of MF-S Cells. (In collaboration with Dr. Guccion, Laboratory Service, WVAH, and Dr. Schechter, Hematology, WVAH)

Electron microscopic analysis of MF-S cells obtained from samples of peripheral blood, bone marrow and lymph nodes are being carried out by Dr. Guccion. These studies have resulted in clinically useful information in identifying malignant cells in these specimens not readily apparent or classified by routine light microscopy. Dr. Schechter's work has shown that the presence of mycosis cells in the peripheral blood is an important prognostic factor.

2.6 Multiple Myeloma and Plasma Cell Malignancies

2.61 Alternating Cyclic Combination Chemotherapy of Multiple Myeloma with Melphalan, prednisone (MP); Vincristine, adriamycin, cyclophosphamide, prednisone (VCAP); BCNU, melphalan, prednisone (BMP). (Dr. Bunn and Senior Staff, in collaboration with Dr. Schechter, Hematology Section, WVAH, Protocol 76-7)

This protocol tests the effect of giving 6 agents, each active individually (with the exception of prednisone) as alternating cycles of different combinations. Twenty-one patients have been entered on this trial. Fifteen are alive, and 6 have died. There have been 5 complete responses documented by bone marrow, and paraprotein examination; 6 partial responses, 6 non-responders, and 4 patients are too early to evaluate. These response rates are not strikingly different from treatment with melphalan-prednisone (MP) alone although two patients who progressed on MP responded to VCAP. It is too early to evaluate the survival of the series.

2.62 Phase II Trial of VP-16-213 in Multiple Myeloma Failing Primary Combination Chemotherapy. (Dr. Bunn and Senior Staff - Protocol 76-7)

The NCI-VA MOB has had considerable experience with this agent in solid tumors, and VP-16-213 has been reported to have activity against other hematologic neoplasms. Effectiveness in multiple myeloma is still unknown.

2.63 Phase II Trial of Hemibody Radiotherapy in Multiple Myeloma Failing primary and Secondary Chemotherapy. (Dr. Bunn and Senior Staff in collaboration with Dr. Glatstein, ROB/DCT - Protocol 76-7)

Radiotherapy is an effective local agent in the treatment of multiple myeloma but there is only anecdotal reports of responses using radiotherapy as a systemic form of treatment such as in hemibody therapy. Such therapy could also have potential effects on the immune system which may benefit the patients in terms of normal immunoglobulin synthesis (see section 2.57) If responses are seen, this modality would be used in the next primary treatment protocol of multiple myeloma.

2.7 Hepatocellular Carcinoma

2.71 Combination Chemotherapy (Adriamycin and Streptozotocin) of Hepatocellular Carcinoma. (Dr. Ihde and Senior Staff - Protocol 76-11)

Patients with unresectable hepatocellular carcinoma are being treated with a combination of adriamycin, shown to have limited activity in this disease in the previous NCI-VA MOB trial, and streptozotocin, a non-myelosuppressive agent which may have activity. Both drugs are given at full therapeutic doses. Serial determinations of serum alpha-fetoprotein levels are being followed through the courtesy of Dr. Robert McIntire, Laboratory of Immunodiagnosis, NCI. In our previous studies we have found that only patients without jaundice and with a good performance status have a reasonable expectation of responding to chemotherapy. Thus far, one of three "good risk" and 0 of 6 poor risk patients have responded; these results do not appear different than those we obtained with adriamycin alone.

2.72 Surgical Debulking Following Combination Chemotherapy of Hepato-cellular Carcinoma. (Dr. Ihde in collaboration with Dr. Fortner, MSKCC)

A patient whose hepatoma was initially limited to the liver at the start of chemotherapy and who responded to that therapy was considered as a possible candidate for curative resection . Following arteriography and peritoneoscopy the patient was operated upon by Dr. Joseph Fortner of Memorial Hospital, New York, and a palliative resection was performed. The patient tolerated the surgery well and remained ambulatory for a period of 7 months.

2.8 Phase I Trials

2.81 Phase I Trial of Dichloromethotrexate (DCM) in Hepatocellular Carcinoma and Other Solid Tumors (Dr. Ihde, F. Pelsor, and Senior Staff in collaboration with Dr. Myers CPB/DCT - Protocol 78-1)

The methotrexate congener DCM is of theoretical interest because it is effective against some methotrexate-resistant animal tumors and because it is much more dependent upon the liver rather than the kidney for metabolism and excretion. A Phase I protocol has recently been approved using a weekly 6-hour infusion schedule. Pharmacokinetic studies will be done by Frank Pelsor in collaboration with Dr. Charles Myers, Clinical Pharmacology Branch, DCT, NCI. The schedule was chosen because of our previous experience with high dose methotrexate infusions, with leukovorin rescue. An eventual goal will be to study DCM in high doses, with leukovorin, using a radioisotope binding assay.

2.82 Phase I Trial of PALA (In collaboration with Dr. Chabner, LCP/DCT - Protocol 78-3)

This interesting new agent with novel biochemical properties, and non-cross resistance with known agents in animal tumor models is an ideal candidate for Phase I trial in Lung cancer. Eligible patients are entered onto the protocol developed and run by Dr. Chabner.

2.83 Phase I Trial of Pentamethylmelamine (Dr. Ihde and Senior Staff - Protocol in preparation)

Pentamethylmelamine is an analog of hexamethylmelamine suitable for parenteral administration. Because of the activity of hexamethylmelamine in human tumor xenographs and lung cancer and the inability of many patients to tolerate hexamethylmelamine because of GI toxicity, the study of this congener is of interest.

2.9 Staging Studies in the Evaluation of the Natural History and Management of Malignant Disease.

2.91 Fiberoptic Bronchoscopy (FOB)

Our studies have revealed that FOB is a useful if not essential technique in the staging and management of patients with SCBC. The senior staff and trained clinical associates of the NCI-VA MOB perform approximately 300 precedures a year. Each procedure entails detailed visual examination, and pathologic examination of bronchial washings and direct biopsy of lesions, areas of prior disease, or blind biopsies. There have been no mortality and no major complications from over 600 such procedures performed in the population of patients with lung cancer on combination chemotherapy protocols and the procedures are often done in the outpatient setting. The results of 77 SCBC patients have been collected and show that the major uses of this tool are 1) to follow response to therapy in the 10% of patients who have evaluable chest disease by FOB but not by chest xray; 2) to ascertain whether residual tumor is present in patients with a complete response to therapy by chest x-ray (patients with complete x-ray response who have residual tumor by FOB relapse at a rate 4 times that of patients who do not); and 3) to detect early tumor progression in the chest (22% of episodes of chest progression were first found only by FOB and not by chest x-ray).

We have also evaluated the usefulness of this technique for assessing the results of chemotherapeutic treatment in non-small cell lung cancer. Results are available in 47 patients. We found that prior to chemotherapy, FOB is more often completely negative than in small cell cancer (21% vs 7%); responses on chest x-ray are confirmed by FOB only 50% of the time and are rarely detected by FOB alone; many episodes of progressive chest cancer found by chest x-ray do not reveal bronchoscopic progression (54%, in contrast to 12% of such episodes in small cell cancer); and over one-third of episodes of chest progression are detected by FOB alone (vide supra for small cell). Thus, the principal utility of this technique during chemotherapy of non-small cell cancer seems to be earlier detection of disease progression, but the therapeutic implications of this knowledge are clearly less than they would be in patients with small cell tumor.

2.92 Peritoneoscopy (Dr. Bunn and Senior Staff, and Drs. Moss, Nugent, and Huberman)

Peritoneoscopy is being used to stage patients with small cell bronchogenic carcinoma, mycosis fungoides-Sezary syndrome, gastric carcinoma and other selected protocol patients where there is question on the status of intraabdominal disease particulary involvement of the liver. Approximately 2-3 procedures are done per week by our staff in the operating room under local anesthesia, and 2nd and 3rd year clinical associates learn how to perform this procedure. To date there has been no mortality, and little morbidity from this procedure.

2.93 Gastroscopy and Other Procedures to Stage Patients with Gastric Cancer. (Dr. Bunn and Dr. Matthews in collaboration with the GI-Hepatology Section, WVAH)

We have found that serial gastroscopy with biopsy and washings for cytology plasma CEA, and gastric CEA are not useful for analysis of response to chemotherapy in gastric cancer, although gastric CEA is a sensitive measure of the presence of gastric cancer and may prove useful as a screening procedure in large trials. Of interest, simple physical examination, upper gastrointestinal series radiographs, performance status and dry body weight were the best indicators of the presence and response of gastric tumor in the chemotherapy trials.

2.94 Computer Assisted Tomography (CAT scans) (Dr. Ihde in collaboration with Dr. R. Dunnick, CC/NIH).

The utility of CAT scans in the initial staging, following the response to therapy, and in detecting disease progression is being evaluated in several diseases in collaboration with Dr. R. Dunnick of the radiology service of the clinical center NIH. Abdominal CAT scans are being used for the staging of SCBC. At autopsy the pancreas, adrenals, and retroperitoneal lymph nodes are involved with SCBC in 40-60% of patients. These areas are difficult to assess during pre-treatment staging. Abdominal CAT scans are being performed on consecutive patients prior to therapy and after 24 weeks on treatment. Six patients have been examined to date. CNS metastases are a major problem in SCBC (see 2.96) and we are studying head CAT scans in

SCBC patients to try to pick up earlier lesions than are detected with normal radionuclide bone scans. Responses are notoriously difficult to evaluate in prostatic carcinoma and we are sequentially following pelvic CAT scans to evaluate the response of tumor in this area to chemotherapy. Similarly, intraabdominal tumor in hepatocellular and gastric carcinoma, are being staged and followed with abdominal CAT scans.

2.95 Bone Marrow Biopsy in Small Cell Carcinoma (Drs. Ihde, Matthews, and Simms).

The implications of bone marrow metastases in patients with small cell lung cancer were investigated in 73 consecutive patients treated on our intensive chemotherapy protocols. Marrow involvement was present in 19% but was found in only one patient who was not shown to have extensive disease by other means. Marked new bone formation and fibrosis were often seen in positive marrow biopsies. During induction chemotherapy, more RBC transfusions were required by marrow-positive patients, but otherwise the toxicity of induction was unremarkable in comparison to other patients. Over 80% of marrow-positive cases had eventual clearing of their marrow biospies on chemotherapy. Complete response rate and survival were slightly but not significantly poorer than in patients with other types of extensive stage small cell lung cancer, (see also section 2.116).

2.96 Radionuclide Brain Scans and CSF Cytology in the Detection of Metastatic Small Cell Lung Cancer. (Drs. Bunn, Matthews, and Nugent)

Initial and serial radionuclide brain scans, as well as initial lumbar punctures with CSF examination including cytologies were performed on a large number of patients with SCBC. However, no positive CSF cytologies were found in asymptomatic patients, and only one positive brain scan was found in serial follow-up of asymptomatic patients (incidence of 4%). We conclude that staging lumbar punctures, and serial brain scans are not useful in asymptomatic patients with SCBC. In contrast, CSF cytologies were highly rewarding in patients with spinal epidural or neurologic signs suggesting leptomeningeal disease and were positive in over 75% of cases. We conclude that CSF cytology should be routinely performed in patients with evidence of CNS metastases to determine or exclude the presence of leptomeningeal disease.

2.97 Lymphangiograms in Prostate Carcinoma and Mycosis Fungoides-Sezary Syndrome (Drs. Ihde and Bunn in collaboration with Dr. R. Dunnick CC/NIH).

The utility of bipedal lymphangiograms (LAG) in the initial staging, response to treatment, and relapse of patients with prostatic carcinoma and mycosis fungoides-Sezary syndrome are being evaluated. These are both diseases known to involve retroperitoneal lymph nodes but have not had LAG's used to follow disease. In addition, the radiologic characteristics of enlarged groin lymph nodes in Mycosis Fungoides are being established.

2.10 Evaluation of Tumor Markers in the Natural History and Response to Treatment.

2.101 Studies of a Lung Cancer-associated Antigen Present in Human Serum.

Serial serum samples prior to and during therapy are being provided to Dr. Robert McIntire, Laboratory of Immunodiagnosis, NCI, on patients with all histologic types of lung cancer. Dr. McIntire is developing a radioimmunoassay for an apparently specific lung cancer-associated antigen. The sensitivity of this assay in detecting residual tumor in patients in response to therapy would be a question of obvious interest.

2.102 Paraneoplastic Syndromes

We are studying the response to treatment of SCBC of the paraneoplastic syndromes encountered in our patients including the inappropriate antidiuretic hormone (SIADH), Cushing's syndrome of ACTH excess and Eaton-Lambert myopathy syndrome. The latter is being studied in collaboration with Dr. J. Hawley, Neurology Service, WVAH. In addition, a bank of pre and post treatment sera is being established. Calcitonin has been assayed in selected patients by Drs. Becker and Silva of the Endocrinology Section, WVAH and found to be correlated with disease activity in SCBC despite the lack of clinical symptomatology associated with calcitonin excess seen with SCBC.

2.11 Clinico-pathologic Correlations (Drs. Matthews and Gazdar and Senior Staff)

2.111 Correlation of Clinical Response with Subtypes of Small Cell Carcinoma of the Lung (SCCL).

Subtypes of small cell carcinoma of the lung have been correlated with response to therapy and survival. Initial subtyping included three groups: lymphocyte-like type (21); intermediate (22) and a combination of these subtypes (21/22). In an atuopsy study of 91 cases treated on three separate protocols using chemotherapy or chemotherapy/radiotherapy, there was no significant difference in response rate in any of the three subtypes. The 21/22 group behaved identically as the 21 subtypes. Although there were fewer complete responders (CR) in the 22 subtype, the partial responses (PR) were equivalent and no statistical differences could be identified. In 64 patients on high dose chemotherapy protocols, similar findings were encountered. Response rates in the three subtypes ranged from 90-97% and actuarial median survival was 9.5 to 10 months. It is concluded that combined 21/22 can be classified as 21 for coding purposes. It is probably significant that 8 of the 64 patients had paraneoplastic syndromes and all of these patients were classified as 21 or 21/22 and none as the pure 22 subtype.

2.112 Correlation of Tumor Bulk, Relapse Sites and Cause of Death in Combined Modality Versus Chemotherapy Protocols for SCCL.

Autopsy material was reviewed on 18 patients treated with high dose chemotherapy/radiotherapy at the NCI-ROB, (in collaboration with Drs. Breteton, Kent, Glatstein) and on 21 patients treated with high dose chemotherapy alone at the NCI-VA MOB. In general, there was a striking reduction in tumor burden in patients on chemotherapy/radiotherapy protocols. Cause of death in this group related to effects of irradiation and/or central nervous system metastases. This mode of therapy appeared capable of sterilizing the lung fields and debulking the primary tumor so efficiently that only minimal chest disease persisted in the field of irradiation. There was a marked reduction in the number of anticipated extrathoracic metastases. The central nervous system (30%) and the liver (22%) were the only significant sites of relapse. In contrast, patients treated on high dose chemotherapy protocols had extensive tumor burden in the lung and mediastinum (100%); the liver was the site of metastases in 81%; the adrenals, 67%; the CNS, 61% and the bone marrow, 57%. Cause of death was related to tumor burden, particularly hepatic, cerebral and cardiac. We note the time of death in the irradiated patients was 5 months after starting treatment while the time of death of chemotherapy alone treated patients was 10-12 months after starting therapy. This could account for some of the differences seen.

2.113 Development of Different Lung Cancer Histology After Treatment with Chemotherapy and Chemotherapy/Radiotherapy.

Striking morphologic changes are noted in one-third of autopsy material in patients subjected to intensive chemotherapy and/or radiotherapy. These changes include conversion of cells to anaplastic large cell types; formation of syncytial multinucleated giant cells with abundant cytoplasm; transformation to carcinoid patterns, squamous cell nests or distinct gland and tubular structures. The squamous cell nests were particularly identified in fields of irradiation. In two patients receiving chemotherapy and radiotherapy, no small cell tumor was identified at autopsy, but only well-differentiated epidermoid carcinoma was found. It is speculated that such transitions may result when sensitive small cells have been effectively destroyed while less sensitive cells or cell clones are stimulated to maturation. Tumor typing based on post-therapeutic biopsy or cytology specimens or an autopsy material may be highly misleading.

2.114 Gastric Cytologies.

One hundred and twenty one gastric washings and/or brushings have been performed on 99 patients with a variety of upper gastrointestinal diseases including carcinoma, gastritis and ulcers. Twelve of twenty-six patients with gastric carcinoma had positive cytology on single or multiple occasions. Three patients with epidermoid carcinoma of the esophagus were diagnosed by this technique. Eight additional patients had washings or brushings highly suggestive of malignancy. Brushings tended to provide more satisfactory specimens than washings. All patients with positive gastric cytology had poorly differentiated infiltrating adenocarcinomas. Three patients with a linitis plastica type of malignancy had either negative or suspicious cytologies. No positive cells could be identified. CEA levels in gastric fluids were elevated in all patients with positive or highly suspicious cytologies. Because of wide variations that occurred in the specimen collection procedures, no evaluation could be made of patients' responses to therapy, via conversion of positive cytology to negative. Atypical epithelial hyperplasia associated with chronic gastritis was a recognizable cytologic entity. Because of specimen collection problems, this phenomenon was recognized in less than one-half of the patients with gastritis by biopsy. We conclude gastric cytologies are not an efficient was of following response to treatment of advanced lesions.

2.115 Histopathologic Correlation of Gastric Carcinoma in Surgically Resected Patients.

A pilot study was begun to evaluate the histopathology of 45 patients who had undergone gastric resection in the VA Surgical Adjuvant Group, in an attempt to correlate histology with the patients' clinical course. Tumors were typed according to the classification of Lauren into intestinal---implying frank grandular proliferation; diffuse--implying a single cell often signet-ring-type, with rare attempts at gland formation; or a combination of these components. The types were compared with three survival patterns: i.e., patients with rapid tumor progression and death; patients with slow tumor progression and death and those patients free of disease within 3 years. Sixty-five percent of the cases studied to date have been typed as intestinal type; 26%, diffuse; and 9%, a combination of the two types. Five patients who survived longer than 3 years were typed as an intestinal type; 5/8 patients with slow tumor progression were also typed as intestinal type. None of the diffuse types were in the survivor group. We conclude there is a definite correlation of histologic type with survival in gastric cancer.

2.116 Histopathologic Correlation of SCCL in the Bone Marrow.

Fourteen patients with SCCL and bone marrow involvement had sequential bone marrow biopsies reviewed. Nine of the 14 had evidence of myelofibrosis and marked new bone formation, associated with the metastases. Five of the patients had focal marrow involvement, with minimal fibrosis. No frankly lytic lesions were identified. Eight of the 9 patients with osteosclerotic bone had dry taps. Touch preps on biopsy material were positive in 12/13 cases in which they were available for interpretation. Osteoblasts and osteoclasts were identified in a significant number of cases in the Wright-Giemsa touch preps. The remodeled bone and marrow fibrosis persisted and in some cases appear more marked during clinical remission. Bone scans correlated well with the presence or absence of metastatic disease, and were not affected by the osteosclerotic bony remodeling (see also section 2.95).

2.117 Development of a Registry of Long Term Survivors in SCBC. (Dr. Matthews and Dr. Minna, in collaboration with Dr. Mugia AD/DCT).

The NCI, under the aegis of Dr. Franco Muggia, has established an international registry to study patients who have survived over 2½ years with small cell carcinoma of the lung. This registry is maintained at the

2.117 Continued

NCI-VA MOB. To date, clinical data and slides have been received on 104 patients. Fifty-three of the patients have had surgical lung resections, either alone (23) or in combination with radiotherapy, immunotherapy or chemotherapy. Thirty-seven patients have received radiotherapy alone (19) or in combination with chemotherapy. Eleven patients were treated with chemotherapy alone. Information is not available on the remainder of the patients. The majority of these patients have had regional disease and diagnosis has been established by mediastinal or bronchial biopsy, lung biopsy or lung resection. Cervical lymph node biopsies were obtained in less than 10% of the cases. Two patients presented with central nervous system disease. The longest survivor, a patient with a lymphocyte-type of small cell carcinoma, received surgery alone and is still alive 16 years later. This study is in its infancy but its potential impact is impressive.

2.118 Correlation of Histologic Subtyping with Survival in Surgically Resected Lung Cancer.

The pathologic data and slides of over 600 patients with carcinoma of the lung, resected in VASAG protocols have been reviewed by the NCI-VA reference pathologist of this branch. Approximately 80% of diagnoses preferred by the 41 contributing VA Hospitals have been verified. Fifty-six percent of the tumors have been classified as epidermoid carcinoma, 3%, small cell carcinoma; 28% adenocarcinoma and 10% large cell carcinoma. Three percent of the tumors have been classified as adenosquamous cell carcinoma. Over two-thirds of the epidermoid carcinomas have been subtyped as well or moderately differentiated; over one-half of the adenocarcinomas have been a significant papillary component. A study is under way to determine the prognostic significance and effect on survival of tumor differentiation, size, location and presence of lymph node metastases in these patients. This study is being done in collaboration with Dr. George Higgins, Chairman of the VASAG, Dr. Thomas Shields, Chairman of the VASAG Lung Committee, and Mr. Robert Keehn, statistician for this group at the National Research Council. The effect of differentiation on survival has not been studied, to our knowledge.

2.119 Lung Cancer Material from Eastern Cooperative Oncology Group

The NCI-VA pathologist acts as the reference pathologist for the chairman of the lung pathology committee of the ECOG. Pathology slides and data have been received to date on over 100 patients entered into a combined ECOG-RTOG protocol treating patients with limited small cell carcinoma. Protocols are being developed to treat extensive small cell tumors. Surgical, radiotherapeutic and chemotherapeutic protocols are in the process of being activated for non-small cell tumors. Slides from these tumors will be reviewed by the reference pathologist and will be forwarded for tertiary review by members of the lung pathology committee. An ongoing attempt is being made to assure that all committee members understand and agree on lung cancer diagnostic criteria. Appropriate forms have been devised. Attempts are being made to retrieve and retrospectively study slides on patients in the cytoxan-methotrexate and hexamethylmelamine-adriamycin-methotrexate arms treating adenocarcinoma and large cell carcinoma. ECOG statisticians report a 30% response rate for the latter arm.

2.120 Prospective study of Histologic Criteria for the Diagnosis of Mycosis Fungoides, Sezary Syndrome and T-Cell Cutaneous Lymphomas.

A blind study has been devised to evaluate histologic criteria for the diagnosis of the above T-Cell diseases. A total of 104 slides are to be reviewed. One-half of these are to be documented cases of mycosis/ Sezary; the other half will consist of chronic psoriaform dermatoses that mimic mycosis fungoides in gross or microscopic appearance. Slides for the study, the clinical histories, laboratory data and diagnoses have been assembled by a clinical assistant in dermatology. Two oncologic pathologists, (M. J. Matthews and A. Gazdar), a dermatopathologist (Dr. Robert Patterson), and a dermatologist, (Dr. Betty Fischmann, of the Medical Service), are to review the sections. A form which asks approximately 46 different questions pertaining to the pathology of the slides has been devised. An NCI statistician was consulted prior to the onset of the study to assure appropriate design. It is hoped that through this study, baselines may be established to distinguish the pre-mycotic, mycotic plaque and tumor phases of these unusual diseases. The correlation of these studies with clinical and laboratory data currently being collected on mycosis/ Sezary patients may provide meaningful data in understanding the spectrum of these diseases and the relationship of some tumor types which spontaneously heal and recur (lymphomatoid papuloses). A study of the dermatopathic lymphadenitis frequently associated with this disease is also anticipated.

2.12 Infectious Disease Studies. (Dr. Fossieck and Senior Staff)

2.121 Monitoring of Infection During Episodes of Severe Pancytopenia in Patients with Solid Tumors Undergoing Intensive Combination Chemotherapy.

Fever is almost a universal occurence in small cell carcinoma patients receiving induction combination chemotherapy, but infection is not. For example, 23 cycles of intensive chemotherapy without modification for hematologic toxicity have been given in the CAPO protocol (77-3). This has resulted in a mean of 11 days per cycle of polymorphonuclear leucocyte count less than 1000/µl. These nadirs have been accompanied by a mean of 6 days/ cycle of fever over 38 C. However, in these 23 cycles there have been only 2 proven infections (infections in 9% of cycles) and no septic deaths. Thus, while we have been routinely given systemic combination antibiotic therapy when fever develops in the severely granulocytopenic patients, the incidence of documented infections appears to be surprisingly low despite intensive culturing and search for bacterial etiologies.

2.122 Gram Positive Coccal Bacteremia in Patients with Neoplastic Disease.

Fifty-seven cases of gram positive coccal bacteremia have been studied. Immediate mortality was 23%. Important etiologic factors include breach of mucocutaneous barriers in 95%, and a poor (prebacteremic) performance status (PS). Prognostic factors include a poor PS (all deaths occurred in patients confined to bed 50% or more of time, PS 3,4), infection with S. pneumoniae (36% mortality), and less than optimal antibiotic therapy (63% mortality). Thirty cases of S. aureus bacteremia have occurred.

Again, cutaneous entry sites are implicated in the majority while metastatic foci of infection have been rare. Effective therapy has been 1 week of intravenous nafacillin with completion of therapy using oral dicloxacillin.

2.123 Bacteremia in Patients with Mycosis Fungoides-Sezary Syndrome

Twenty-four episodes of bacteremia in patients with mycosis fungoides-Sezary syndrome have been studied. This is a major problem in the management of these patients with combined modality electron beam and chemotherapy. Nine percent of these infections have been due to S. aureus. There were no immediate deaths related to staphylococcal infection, but one death occurred in septicemia with PS. aeruginosa.

2.124 Herpes Zoster in Patients with Solid Tumors.

Herpes zoster infections have occurred in 10% of patients with small cell carcinoma treated with intensive combination chemotherapy. This incidence is 10-fold greater than previously noted in solid tumor patients. Most cases occur during tumor remission.

2.125 Pneumocystis Carinii and Other Opportunistic Infections in Patients with Solid Tumors.

Pneumocystis carinii pneumonia has occurred in 4 patients with lung cancer (2 receiving corticosteroids and 2 not on steroids). Other opportunistic infections with protozoal, and viral disease have also been found. These infections have not been previously recognized as complications of solid tumors, and undoubtedly reflect impairment of host defense mechanisms associated with intensive chemotherapy.

2.126 Bacteremia During Staging Procedures.

The occurence of bacteremia in patients undergoing staging bronchoscopy and peritoneoscopy is being studied in collaboration with the pulmonary and gastroenterology sections. The studies to date show no transient bacteremia associated with these procedures.

2.127 Bacteriology of the Lower Respiratory Tract in Patients with Lung Cancer.

Cultures of sputum and translaryngeal fiberoptic bronchoscopic aspirates have been studied in 31 afebrile lung cancer patients prior to chemotherapy. The results show sputum cultures and FOB specimens do not provide accurate bacteriologic surveillance data about microorganisms present in the lower respiratory tract of these patients. These studies are of importance in planning appropriate antibiotic therapy for infections which develop during intensive chemotherapy.

2.128 Clinicopathologic Correlates of Candida Infections in Cancer Patients. (Dr. Ihde in collaboration with Dr. Brereton ROB/DCT).

Clinical records and autopsy protocols of 168 consecutive cancer patients dying with histologically-proven candida infections were studied at the Clinical Center, NIH, in collaboration with Drs. Harmar Brereton, Radiation Oncology Branch, DCT, NCI, Robert Young, Medicine Branch, DCT, NCI, William McGuire, Cancer Therapy Evaluation Program, DCT, NCI, and William Roberts, Pathology Branch, NHLBI. Clinical and pathologic features predictive of local and disseminated infection in this population were determined. The clinical and pathologic findings in 17 patients with cardiac candidiasis were studied in detail and contrasted with those found in candida endocarditis.

2.129 Miscellaneous

In collaboration with the pulmonary section (Dr. Spagnola) we have prepared a case report describing a case with cure of localized pulmonary phycomycosis. This disease is usually fatal and an approach to management which includes CAT scanning, surgery and perioperative amphotericin B is described.

In collaboration with the infectious disease section (Drs. Parker and Kane) we have reported on two cases of _Hemophilus parainfluenze_ endocarditis in drug users, including a discussion of the pitfalls in diagnosis and the appropriate therapy.

2.13 Hepatitis Virus (In collaboration with the GI-Hepatology Section, Drs. Seef and Hoofnagel).

2.131 A prospective Study of Hepatitis Virus Transmission by Fiber-optic Bronchoscopy) (FOB).

We have investigated the possible transmission of hepatitis B virus by (FOB). Serial serum samples taken just before and periodically after FOB were obtained in 98 consecutive patients. None of these patients, were HB_s Ag positive prior to introduction of the FOB and none converted thereafter.

2.132 Prospective study of Hepatitis Virus Transmission in an Oncology Unit.

A prospective study of hepatitis virus transmission in an oncology unit has been started. The study involves systematic monitoring of all patients and staff for both hepatitis virus antibody and antigen and correlation of positive studies with various procedures and transfusion history. To date, the results have revealed no transmission of hepatitis in the unit.

2.14 Diagnosis and treatment of cardiac tamponade in patients with malignant disease. (Drs. Simms and Minna in collaboration with Drs. Katz, DiBianco, and Fletcher of the Cardiology Section, WVAH.)

14 patients with lung cancer had hemodynamic studies at cardiac catheterization and then conservative treatment with pericardial tap and tube drainage and chest radiotherapy for symptomatic pericardial tamponade. The tamponade was documented with echocardiogram and at the time of catheterization with equalization of diastolic pressures, a paradoxical pulse greater than 10 mm Hg, equalization of right atrial and intrapericardial pressures. We found that severe impairment of cardiac output and hemodynamics were detected at catheterization that were not apparent by routine physical examination; that malignant cytologies were found in a high percentage (10/14), allowing the diagnosis to be established; and that with improvement of post-tap hemodynamics, there was infrequent need for a second tap or pericardial window. The large majority of patients did not have relapse of hemodynamic symptoms. We conclude that histologic diagnosis, symptomatic and hemodynamic relief can be achieved by this conservative approach.

2.15 Interstitial Lung Disease in patients with Solid Tumors receiving intensive chemotherapy. (In collaboration with Drs. Spagnolo, Pulmonary Section WVAH and Dr. Schafer, Thoracic Surgery, Surgical Service WVAH and Dr. Ortega Laboratory Service WVAH).

30 episodes of interstitial pneumonitis have been studied in patients with solid tumors undergoing intensive combination chemotherapy. Routine procedures, transbronchial biopsy and open lung biopsy have been used to establish a diagnosis. Drug toxicity, protozoal infections, viral disease, and interstitial lung disease of unknown etiology have been found. These cases are being studied for long term followup and analysis of resultant pulmonary dysfunction.

2.16 Development of Computer Programs for Clinical Studies. (Dr. Minna Dr. Matthews, Dr. Moss and Mr. L. Berman,private consultant).

A set of interacting programs for storing and retrieving clinical research data, laboratory data, data on pathologic slides, drugs received, and referring physicians has been completed. The program is being tested and personnel trained to input clinical research data into the computer terminal which is linked up with the NIH computer facilities. In addition, a PDP 11 computer has been purchased but not yet delivered, and we are awaiting various clearances in VACO. This computer will be used for storing both clinical and laboratory research data on site, and should facilitate batch processing data to the NIH computer. This interaction with the NIH computer facilities is difficult at this time because of overusage of the facilities during regular working hours.

2.17 Literature Reviews of Malignant Disease (Senior Staff).

2.171 Several reviews of the management of small cell bronchogenic carcinoma has been prepared for publication.

2.172 A review of current therapy of Mycosis Fungoides-Sezary Syndrome has been prepared for publication.

2.18 Oncology Nursing (M. Boyer and nursing staff).

The nursing staff of the NCI-VA M.O.B. in addition to their regular duties are contributing on both a national and local level to improve the nursing care of cancer patients.

2.181 Lung Cancer. Marjorie Boyer wrote an article "Treating Invasive Lung Cancer" which was published in the American Journal of Nursing. J. Eddy and M. Boyer each spoke several times to the Georgetown Oncology Nurses Association on the care of the person with lung cancer.

2.182 Mycosis Fungoides-Sezary Syndrome.

These patients have tremendous problems with skin care, erythroderma, staphylococcal septicemia from skin infections, and burns from combined modality electrobeam and chemotherapy. Several nurses are working on an article summarizing the specialized nursing care of these patients.

2.183 National Organizations.

R. Selgas is an officer of the National Oncology Nursing Society and the local Association of Washington Area Cancer Nurses (AWACN). Some of the nurses are active members in both organizations.

2.19 Research Pharmacy Activities. (Pelsor, Lee in collaboration with CPB/DCT.)

Interpatient variation of drug disposition has long been recognized as an important component of response to drug therapy. Until recently, rapid and specific methods for analysis of biological fluid concentrations of antineoplastic drugs have not been available. Now, plasma, urine and CSF concentrations of drug may be monitored in order to develop optimal dose schedules in each patient. Further, elevated concentrations of drug may indicate ensuing toxicity which may not be apparent clinically. With this aid the physician may begin appropriate antidotal therapy and possibly avoid severe toxicity.

At NCI-VA MOB we use a competitive protein binding assay to monitor patients who receive methotrexate. Plasma concentrations are used to guide the administration of "leucovorin rescue." This procedure permits the administration of high doses of drug which might otherwise be toxic. We will monitor dichloromethotrexate similarly. In addition, an assay procedure for adriamycin and its metabolites is being developed.

This information will be valuable in monitoring adriamycin therapy in those patients who have hepatic and/or renal dysfunction. Furthermore, frequent serial samples of several biological fluids may be used to develop kinetic models of drug disposition. We have developed such a model for the epipodophyllotoxin derivative, VP16-213, which will aid in planning new dosage regimens for this drug. These studies are being done in collaboration with the Clinical Pharmacology Branch(DCT Drs. Chabner and Myers).

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PROJECT NUMBER

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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Laboratory Investigation of Tumor Cell Biology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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NCI-VA Medical Oncology Branch

SECTION

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INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
20	9	11

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project uses a multidisciplinary approach to study tumor cell biology, so as to further our understanding of the basic nature of human malignancy, and to develop techniques for the early detection and better methods of treating human cancer. Our major efforts are in growth of human tumors in vitro and in nude mice to study the differentiation, cell kinetics, immunology and immunotherapy, and experimental chemotherapy, radiotherapy of these model systems. Another major area is the use of somatic cell hybrids to study tumor cell biology and cell genetics. These include genetic studies of: mouse enzymes for comparative gene mapping; human enzymes; type-C viral replication (primate tropic and MuLV ecotropic and amphotropic), viral receptors, integration sites; production of monoclonal antibodies using hybridomas against defined proteins, and normal and lung cancer cell antigens; study of tumor blood flow, cell kinetics of patient samples; cellular immunology of human lymphocytes against autologous tumor in vitro, and immunologic methods for the early diagnosis of infection.

PHS-6040
(Rev. 10-76)

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3.0 LABORATORY INVESTIGATIONS OF TUMOR CELL BIOLOGY.

3.1 Overview.

From the clinical study of patients with malignant disease and their treatment trials the investigators of the NCI-VA Medical Oncology Branch have developed questions which they are asking in the laboratory. In addition, they are attacking fundamental problems of cell biology the answers to which can eventually be used to control tumor cells in patients. We have first been attempting to establish in vitro and in vivo (in athymic nude mice) human tumor cell lines from our own patients or collected elsewhere of the types studied in the clinical trials (lung, gastric, prostate, hepatocellular, mycosis fungoides, multiple myeloma). We have paid particular attention to small cell lung cancer, mycosis fungoides (T-Cell Lymphoma), and multiple myeloma (plasma cells). It is particularly important that these cells come from untreated patients, be free of mycoplasma contamination, or contamination with other cell lines, and any type-C viral contamination. We are thus concentrating on fresh material from untreated patients. We attempt to establish clonal derivatives, study their ability to differentiate, determine their growth factor and hormonal requirements, and study their sensitivity to chemotherapy, radiation therapy, and immunotherapy in vitro and in the nude mouse model system. Model systems for studying animal tumors for tumor blood flow oxygenation, and cell kinetics, have been developed and will be applied to the human tumor xenographs. The plan is to develop assays for cloning tumor cells directly from patient samples and thus be able to determine the clonigencity before and after treatment; 2) to use the growth factor requirements and agents inducing differentiation to establish new methods of treating patients; 3) to use the results of in vivo tests of the human xenographs to design better chemoradiotherapy models; 4) to use the in vitro autologous lymphocyte responses to define cellular immunity of the patients against these tumors.

Our laboratory is particularly experienced in somatic cell hybridization techniques. We have been using this method to; - map mouse, human, rabbit and Chinese hamster genes for comparative genetic studies; by fusion of normal mouse cells with human lung cancer lines, or other malignant cells, we have been attempting to identify human genes which generate malignancy, and mouse genes which suppress malignancy. Our laboratory also has considerable experience with cell biologic studies of type-C viruses, and we have been combining these with somatic cell hybrid methods to identify human and mouse genes required for type C viral replication. A new hybrid cell technique for generating monoclonal antibodies in tissue culture (hybridomas) is being used to generate specific antibodies against defined proteins, human lung cancer cells, and human and mouse lymphocytes. These reagents should be of use in both clinical and cell biologic studies.

Techniques for studying cell kinetics in vitro and in vivo both in humans and in nude mouse xenografts have been established and we are systematically examining the kinetics of our human tumor cell lines.

Techniques for monitoring in vitro lymphocyte responses against autologous tumor cells, and to grow functional human T cells in vitro have been established. We are trying to use these to affect in vitro sensitization of human lymphocytes against lung cancer antigens which could be used in passive adoptive immunotherapy, and to follow the effect of patient treatment on cell mediated immunity against autologous tumor antigens in the patients on protocol treatment.

3.2 Culture of Human Tumors In Vitro. (A. Gazdar, H. Sims, E. Russell)

3.201 New Models for the Study of Lung Cancer

Much effort has been expended in developing new models to study lung cancer. Tumor tissue from cancer patients is explanted into athymic nude mice and also established as permanent cell culture lines. While most of the work has concentrated on small cell carcinoma of the lung (SCCL), other lung tumor lines, including adenocarcinoma, large cell undifferentiated carcinoma, and mesothelioma lines have also been established. We have found approximately 25% of SCCL tumors can grow progressively in nude mice, but only about 10% can be established as permanent cell lines. We have established 4 transplantable SCCL lines in nude mice, and are characterizing 3 others obtained from other sources. After subcutaneous innoculation, most SCCL lines grow as expansile non-invasive lesions and do not metastasize. The same lines innoculated intracranially grow diffusely in the subarachnoid space, or as invasive, destructive intracerebral tumors. Histologically, all of the lines have an intermediate SCCL morphology (WHO 22), and do not resemble the small lymphocyte like (oat cell WHO 21) subtype of SCCL. In culture, SCCL cells may assume a large variety of morphological forms, including polygonal or elongated cells, small cells that pile up, or cells that float in large aggregates or as ribbon like cords. Several lines have been maintained for periods of 7-12 months, and 3 are regarded as established permanent cell lines. The lines are hypotetraploid, double every 1-4 days, and clone at low efficiencies in liquid and semi-solid media. They induce tumors in nude mice of intermediate SCCL morphology. The transplantable and in vitro SCCL lines have been tested for APUD characteristics. They secrete polypeptide hormones, have neurosecretory granules, and have formaldehyde induced fluorescene which is much more brilliant after 5 hydroxytrptophan incubation, but not after incubation with DOPA. These experiments suggest that the cells selectively take up amine precursor (5-HT but not DOPA), decarboxlate it to serotonin, and store it, in the form of neurosecretory granules.

3.202 New Models for the Study of Lymphomas

While many EBV transformed B cell lines from normal patients and those with lymphomas have been established, relatively few T cell lines exist, and none from cutaneous T cell lymphomas. We have attempted to establish such cell lines from lymph nodes and peripheral bloods of protocol patients with mycosis fungoides or Sezary's syndrome. Three permanent fast dividing cell lines have been established from the nodes of 3 patients with lymph node involvement. However, all three lines have one or more of the following B cell markers - surface immunoglobulin, EBV virus or C' receptors. All 3 lines are tumorigenic when innoculated intracranially into nude mice.

A recent report has suggested that normal T cells require factors released by stimulated T cells for growth. We have prepared such stimulated supernatant fluids, using pooled lymphocytes from normal human volunteers. Both the concentrated fluids as well as concanavilin A stimulate the growth of E rosetting cells from mycosis fungoides patients. These cells have been maintained in culture for relatively short periods, and it is not known whether they will become established cell lines. Their growth continues to be factor dependent, cell death occurring in its absence. Apart from lymphocyte surface markers, further characterization of the cells has not been performed.

3.3 Growth of Human Tumors in Nude Mice. (A. Gazdar, H. Sims).

Athymic nude mice provide useful models for the heterotransplantation of human tumors. We have innoculated over 100 human primary and metastatic tumors into nude mice. In these studies we find, gastrointestinal tumors and melanomas have high take rate; sarcomas, lung cancer, germ cell tumors intermediate take rates; and leukemias, lymphomas, endocrine secreting or dependent tumors are exceedingly hard to grow. The tumor inducing inoculum varies from 1 x 10^5 to 3 x 10^7 cells. Subcutaneous (SC) tumors tend to grow as expansile non-invasive tumors. However, some sarcomas, and lung tumors are locally invasive. Intracerebral (IC) innoculation results in diffuse meningeal growth or invasive, locally destructive tumor masses. A smaller number of cells is required for IC growth, and the latent period is shorter than after SC injection. Intravenous or intraperitoneal innoculation has been unsuccessful. Another remarkable feature is the absence of metastases. Only a single hepatic metastasis has been noted in over 120 mice autopsied. Lymphomas and related malignancies do not grow subcutaneously but some grow intracranially. We have grown B and T cell lymphomas, EBV transformed "normal" lymphocytes, and myeloma after intracranial innoculation. The take rate is higher if the cells are first grown in tissue culture. Tumors grown in nude mice frequently maintain the same level of histologic differentiation as the original tumor, even after multiple transplants. Gram quantities of tumor tissue are available. Chemotherapy and radiotherapy studies are possible. However, contamination with murine leukemia virus, xenotropic strain, occurs frequently (40% of transplanted tumors) and poses an unknown biohazard, as well as alters the _in vivo_ growth rate and immunogenicity of the tumor cells.

3.4 Genetic Analysis of Human Lung Cancer using Hybrid Cells (Carney, Edgell, Gazdar, Minna)

Hybrid cells offer unique model systems to study the genetic abnormalities in malignant cells. The data from intraspecies hybrids is conflicting and confusing, partly because the chromosome segregation patterns cannot be analyzed. We have used interspecies hybrid cells to determine whether the malignant or non-malignant phenotype is dominantly expressed. If the malignant phenotype is dominant, and if the chromosomes of the malignant partner are being segregated, the chromosome/s associated with malignancy may be identified. We have used athymic nude mice to test the tumorigenicity of the hybrids, and have compared tumorigenicity with another commonly used *in vitro* indicator of malignancy, agarose clonability. The human lung adenocarcinoma cell A549 is highly tumorigenic in nude mide, and clones efficiently in semi-solid media. A thioguanine resistant subline of it was developed and fused to bromodeoxyuridine resistant 3T3-4E mouse fibroblast cells. The latter are contact inhibited, non-tumorigenic, and unable to replicate in semi-solid media. Fourteen hybrid clones were selected from HAT supplemented medium. All of the hybrids were non-tumorigenic, but 6 of 14 formed colonies at low efficiency in soft agarose. Isozyme studies indicated that all hybrids were retaining mouse while segregating human chromosomes. While tests for human chromosomes 3,8,22 and Y were not available, all other human chromosomes were present in 3 or more hybrid clones. Thus, we conclude in this particular cross, the non-malignant mouse phenotype is dominant over the malignant human phenotype (although a remote possibility exists that one of the chromosomes not tested for was associated with malignancy, and had been segregated from all the hybrids). In addition, all human chromosomes tested segregated independently of ability to clone in agarose. These experiments also indicate that various parameters of the malignant state can segregate independently in hybrid cells, and that nude mouse tumorigenicity may be the best indicator of the malignant phenotype.

3.5 Genetic Analysis of Mouse Genes from Normal Cells Which Can Suppress Malignancy using Somatic Cell Hybrids, (Moss, Francke, Minna))

Somatic cell hybrids between malignant Chinese hamster cells and normal mouse spleen which segregate the chromosomes of the normal (mouse) parent have been produced and are being studied to determine the correlation between chromosomal complement and the expression of malignant properties. Studies to date have shown that no single mouse chromosome (excluding chromosome #11 which is present in none of these hybrids) can suppress the growth of these hybrids as tumors in nude mice.

3.6 Growth Factor and Hormonal Requirements for Small Cell Carcinoma of the Lung (SCCL) *In Vitro*
(Simms, Gazdar, Minna)

In a patient, SCCL proliferates rapidly, metastasizes widely, and is almost always fatal. Despite this highly malignant behavior in the patient, relatively few attempts at establishment of permanent culture lines have been successful (about 10%). Many cell cultures have been maintained for periods of over 12 months with only minimal growth. Attempts to separate the tumor cells from accompanying non-malignant cells often leads to the death of the SCCL cells. Addition of conditioned medium from other SCCL lines aids the initial growth of the tumor cells. These observations suggest that SCCL cells have special growth requirements, and may be dependent on factors released by other cells for their growth. We are currently attempting to supplement the growth medium of SCCL cells with all of the available hormones and factors known to influence cellular division. In addition, we have been systematically trying to grow SCCL in completely defined serum free, hormone containing media as determined by Dr. G. Sato. These methods may lead to the identification of the special factors required for the growth of SCCL cells, and may eventually lead to methods of controlling tumor cell proliferation in the patient.

3.7 Cloning of Mammalian Genes for Insulin in Hybrid Cells.
(Oie, Gazdar, Minna) (and William Chick, Harvard Medical School).

From other hybrid cell work, a special principle that emerges is that differentiated functions are maintained in hybrid cells if the fusion partners are of similar embryologic origin. Thus, we are trying to use hybrid cells as cloning vehicles for differentiated functions.. Establishment of *in vitro* cell cultures that produce specific polypeptide hormones would provide sources for the latter as well as help us to understand the physiological control of hormone secretion. We have selected insulin production as our model, although the same methodologies could be applied to any hormone. Insulin secreting cells (possibly of neuroectodermal origin) cannot be maintained in long term culture, despite intensive efforts by several laboratories. Through Dr. William Chick, we have obtained a high insulin secreting rat islet cell tumor.. After Ficoll gradient centrifugation, over 90% of the cells are beta cells. We have fused these cells with mouse neuroblastoma cells. Of the 15 hybrids so obtained, all retained their neural properties, but none released insulin or had recognizable beta granules. The hybrids retained mouse while segregating rat chromosomes. Most of the hybrids were fairly well segregated before they could be tested. Either the mouse genome suppressed the production of insulin, or the rat chromosome(s) necessary for insulin production had segregated prior prior to testing. We are currently preparing and testing several other hybrid combinations in an effort to identify an insulin producing combination. Attempts are being made to retain rat while segregating the chromosomes of the other partner. Other parent cells include cells embryologically closely related to islet cells and human tumor cells known to produce ectopic hormones.

3.8 Identification of Mouse Chromosomes Required for Replication of Murine Ecotropic and Amphotropic Type C Viruses, and Virus Specific Cell Surface Receptors using Somatic Cell Hybrids.

Requirement of Mouse Chromosome 5 for the Expression of Murine Ecotropic Virus-Specific Cell Surface Receptor.

(Oie, Gazdar, Lalley, Francke, Minna)

Our work has shown that mouse chromosome 5 is required for mouse ecotropic virus replication, and that mouse chromosome 8 (or 19) is required for mouse amphotropic virus replication. The replication patterns of murine ecotropic and amphotropic virus strains and the binding of Rauscher leukemia virus envelope glycoprotein (RLV gp71) were studied in 18 hamster x mouse hybrid clones, segregating mouse chromosomes. Binding of RLV gp71 correlated with ecotropic virus replication but not with amphotropic virus growth, suggesting that all ecotropic viruses use a common cell surface receptor which differs from that used by amphotropic viruses. A comparison of the mouse chromosome segregation patterns (as determined by isozyme and chromosome analyses) of the hybrid clones which retained the gp71 binding ability with those clones which had lost it showed that mouse chromosome 5 was necessary for RLV gp71 binding by hybrid cells. All other chromosomes were discordant with their phenotype by enzyme or chromosome analyses. One clone which did not bind RLV gp71, lacked peptidase S but retained phosphoglucomutase-1 (both enzymes are assigned to mouse chromosome 5), suggesting that the gene(s) required for the expression of virus specific cell surface receptor was located in the portion of chromosome 5 containing the gene for peptidase S. Thus, Rec-1, the mouse gene previously shown to be required for ecotropic virus replication and assigned to chromosome 5 may determine the expression of virus specific cell surface receptors necessary for ecotropic virus absorption to mouse cells.

3.9 Identification of mouse chromosomes required for spontaneous expression of endogenous ecotropic and xenotropic murine leukemia virus in somatic cell hybrids. (Park, Gazdar, Lalley, Francke, Minna).

We have examined the spontaneous expression of endogenous murine leukemia viruses (MuLV) of the ecotropic (N or B) and xenotropic (X-tropic) classes in Chinese hamster x mouse hybrid cells. We found that MuLV expression was mouse strain dependent. All cultures of AKR parental spleen cells and 5 of 7 hybrids expressed N-tropic virus. All cultures of NZB parental spleen cells and 5 of 8 hybrids expressed X-tropic virus. However, none of 13 BALB/c parental spleen cultures expressed virus, while 6/20 hybrid clones expressed N-tropic or N and X-tropic viruses. These results suggest segregation of mouse regulatory genes in the BALB/c hybrid cells. At present no individual mouse chromosome can be assigned this regulatory function. In the hybrids expressing N or X-tropic virus we have determined the isozyme and chromosome composition to try to correlate the presence or absence of specific mouse chromosomes with viral expression. In AKR somatic cell hybrids we have found that mouse chromosome 7 was required for N-tropic virus expression. A major structural locus (Akv-1) for N-tropic virus has been assigned to chromosome 7 in mice by sexual genetics and we believe we have seen this effect in somatic cell hybrids. However, we have found that continued virus expression can occur independently of chromosome 7 in AKR hybrid cells suggesting this chromosome is not needed for the maintenance of viral expression. In BALB/c somatic cell hybrids we have found chromosome 7 is necessary but not sufficient for initial virus expression suggesting that other chromosomes are needed in this mouse strain. We have found that spontaneous expression of mouse X-tropic virus in NZB hybrid cells was syntenic with mouse chromosome 2 suggesting that a mouse gene for X-tropic virus expression can be assigned to this chromosome. However, a relatively small number of NZB hybrid cells has been studied to date, and only single discordant clones have so far been found for mouse chromosomes 1,3,10,19. Thus, a firm gene assignment awaits analysis of larger numbers of hybrid clones. In these studies, we have also found that N and X-tropic virus expression does not require chromosomes carrying other genes related to MuLV metabolism: Fv-1 (chromosome 4); Fv-2 (chromosome 9); Rec-1 (chromosome 5); and Gv-1 and H-2 (chromosome 17).

3.10 Identification of Human Chromosomes required for Replication of Primate Tropic Type C Viruses in Somatic Cell Hybrids. (Brown, Oie, Gazdar, Francke, Minna).

Two approacheshave been utilized to determine the human genes required by the endogenous baboon M7 virus and the Wooley monkey SSAV virus for infection of rodent x human somatic cell hybrids. In the first approach, hybrid clones were exogenously infected with M7 or SSAV. This approach examines the entire infection process from virus attachment to virus release. In the second approach, cloned, virus infected human cells were fused with rodent cells to produce hybrid clones. By this method, only virus replication events subsequent to virus genomic intergration into cell nuclear material can be studied. This approach is discussed under 3.11.

In both cases, human chromosomes are lost, while rodent chromosomes are retained. By correlating virus expression with the human chromosome content of each hybrid clone, human genes required for virus infection and replication can be identified.

3.101 Baboon Endogenous M7 Virus

The replication pattern of the endogenous baboon type C virus M7 was studied in Chinese hamster x human hybrid cells. The M7 virus replicated in human but not Chinese hamster cells. Twenty-nine primary clones infected with the M7 virus, and 27 subclones derived from 2 of the infected primary clones, were analyzed for virus replication, isozyme expression, and chromosome composition. Only chromosome 19 was found to be concordant for M7 virus infection. All other chromosomes segregated discordantly from M7 virus infection. The virus produced by hybrid cells was identified as the M7 virus by its host range and its reaction with virus group specific fluorescent antibody. In these hybrid cells, human chromosome 6, previously reported to be necessary and sufficient for M7 replication in other rodent x human hybrid cells, was not sufficient by itself but may be necessary for M7 replication.

3.102 Wooley Monkey SSAV Virus

Twenty-two Chinese hamster x human hybrid clones were infected with SSAV and analyzed for virus replication and isozyme expression. SSAV replicates inefficiently in hamster cells creating problems in the analysis of SSAV infection of these hybrid cells. Nevertheless, the infected clones can be grouped into two classes: those clones which are poorly infected (2% of cells virus +, like parent Chinese hamster cells) and designated negative for infection, and the hybrid cells which were efficiently infected (20-100% virus +) (considered positive). When analyzed for virus infection and isozyme expression under these conditions, none of the isozyme markers for 19 chromosomes segregated with SSAV infection. Hence, SSAV and M7 virus replication require different cellular genes, since neither chromosome 6 nor 19 were found to be necessary for SSAV replication. Since isozyme determinations could not be done for chromosome 3, 16, 17, 22 and Y, karyotyping is being done to determine whether these chromosomes might be required for SSAV infection. There is also a possibility that functions determined by genes on multiple chromosomes may be necessary.

3.11 Identification of Human Integration Sites for Primate Tropic Type-C Viruses using Somatic Cell Hybrids. (Gazdar, Oie, Edgell, Russell, Minna).

Attempts have been made to determine the human cellular intergration sites of primate tropic typeC viruses using somatic cell hybrids. Chronically infected clones of the permissive human lung adenocarcinoma cell A549 in which every cell should carry the intergrated virus genome in the same human chromosome(s) were fused with rodent cells absolutely or relatively resistent to infection. All of the hybrid clones obtained segregated human while retaining rodent chromosomes. It was presumed that continued expression of virus was dependent on retention of the human chromosomal intergration sites(s).

3.111 Baboon Endogenous M7 virus

Human A549 cells were preinfected with M7 virus, cloned, and a virus positive clone fused to mouse 3T3-4E or hamster BHK cells (both TK minus). A large number of primary and secondary clones were tested and all of the mouse x human, and most of the hamster x human hybrids expressed the M7 virus. In many cases the level of virus expressed was low, and required co-cultivation for detection suggesting regulation of the virus intergrated into human chromosomes by rodent genes. Isozyme markers for all human chromosomes tested (markers on chromosomes 3, 8, 22 and Y were not tested) were absent in at least some of the hybrid clones made with mouse cells. In addition, since chromosome 17 was selectively retained in this system, the hybrid clones were back selected in BrdU to lose this chromosome; however, these hybrids still expressed M7 virus. This inability of mouse x human hybrids to segregate M7 virus production led to a re-examination of the seeming non-permissiveness of mouse cells to M7 exogenous infection. Using high titer M7 preparations and amplification techniques for testing, mouse cells could be productively infected in all cases. In addition, if human preinfected cells were co-cultivated with mouse cells, and the latter selectively cloned, all mouse clones so obtained were positive for virus. These experiments indicate that mouse cells are only relatively resistant to infection, and this resistance appears to be at a post intergration level. Thus, our experiments suggest interspecies transfer of virogene occurs in hybrid cells. In the hamster x human hybrids several M7 virus negative, and many M7 virus positive clones were found. Isozyme analysis of these hybrids excludes their assigned region on the following human chromosomes as being sufficient as the human intergration site of M7 in A549 cells: 4,5,6,9,11, 12,13,14,15,16,17,18,19,20,21, and X. While certain rodent human hybrids are unsuitable for gene mapping, we are continuing to try to establish specific intergration sites in the hamster x human hybrids segregating the M7 virogene(s).

3.112 Wooley Monkey SSAV Virus

Human A549 cells were preinfected with SSAV, cloned, and a virus positive clone fused to mouse 3T3-4E or Syrian hamster BHK cells. A large number of hybrid clones were tested, and all clones retaining human markers expressed viral group specific antigen. Since human chromosome 17 is selectively retained in these hybrids, it was selected against in BrdU supplemented medium. However, all these hybrid clones continued to express SSAV virus suggesting that human chromosome 17 was not the intergration site. Isozyme analysis showed all other chromosomes to be absent in at least some cases. Several primary clones expressed only 30% positive cells by fluorscent antibody tests and these were subcloned. The secondary clones could be divided into virus negative and positive classes. Thus, it was possible to segregate the SSAV virogene in these hybrids. These results suggest several human chromosomes may serve as the intergration site for SSAV, and isozyme analysis of these positive and negative subclones should aid in identifying the chromosomes

3.113 Feline Xenotropic CCC Virus

A549 cells were preinfected with feline xenotropic CCC virus and a virus positive clone fused with virus resistant mouse 3T3 cells. Virus positive and negative hybrid clones were found. DNA polymerase levels in the virus positive clones were 10-1000 fold lower than in the human parent suggesting regulation of CCC virogene(s) by mouse chromosomes. Isozyme markers for all human chromosomes tested (3,8,22, and Y were not tested) segregated independently of CCC virus production. Further studies are required to determine if a combination of chromosomes, several different chromosomes or the untested chromosomes are required for virus release. However, because amplification methods are nesessary for viral detection we are stopping work on this system.

3.12 Mapping of Human Genes Using Somatic Cell Hybrids. (Brown, Lalley, Francke, Minna).

Our laboratory has developed a large series of primary clones of mouse x human and hamster x human somatic cell hybrids segregating human chromosomes from fusions with several different human cell lines. These hybrids are useful for mapping human genes. We have established assays for 35 different human enzymes and most of these have been performed on the primary hybrid clones. Several of the isozymes are known to be syntenic and our hybrids also show this relationship and thus provide additional confirmation of these gene assignments provided by other labs. Thus, we have a bank of hybrid cell lines and hybrid cell homogenates that have been characterized for their human gene content. These hybrids have been primarily used for analysis of type C virus genetics (see section 3.10). However, we have also used them to assign a human gene for Peptidase S to human chromosome 4, and to confirm the assignment of human Peptidase D to human chromosome 19. We will maintain these hybrids in frozen cell storage for future studies in our lab and provide them as reference lines to other interested investigators. Dr. Francke has karyotyped approximately 15 of the primary Chinese hamster x human somatic cell hybrids and several with interesting chromosome rearrangements or deletions have been added to her panel for subchromosomal gene localization.

3.13 Mapping of Mouse Genes Using Somatic Cell Hybrids. (Lalley, Francke, Minna)

We have spent considerable effort developing Chinese hamster x normal mouse spleen somatic cell hybrids segregating mouse chromosomes that can be used for mouse gene mapping. This project now extends over 5 years. The purpose was to map mouse genes whose human homologues had been also mapped in man and then to compare the mouse and human gene synteny maps. A panel of 50 primary clones was established, and 25 of these had chromosome banding studies performed in detail by Dr. Uta Francke of the Dept. Pediatrics, University of California, UCSD, La Jolla in a collaborative effort. The following mouse enzymes have thus been mapped using our somatic cell hybrids: chromosome 1, ID-1, DIP-1; chromosome 2, AK-1; chromosome 4, PGM-2, AK-2, ENO-1, PGD; chromosome 5, PGM-1, PEP-S; chromosome 6, TPI-1; chromosome 7, GPI-1, PEP-D, LDH A, ID-2; chromosome 8, GR-1, APRT; chromosome 9, MPI-1, PK-3, MOD-1; chromosome 10, HK-1, PP, TRIP-1; chromosome 12, ACP-1 chromosome 14, NP-1, ES-10; chromosome 16, SOD-1; chromosome 17, GLO-1; chromosome 18, DIP-2; X chromosome, A-GAL, HPRT;and with human x mouse hybrids losing mouse chromosomes, G6PD.

(The work on TPI-1, GLO-1, and SOD-1 was done in collaboration with Drs. G. Bruns and P. Gerald of Genetics Division, Dept. Pediatrics, Harvard Medical School).

In addition, to the new gene assignments, the genes that had also been mapped by sexual genetics provide confirmation of the correction of the scoring of mouse chromosomes in the hybrid cells, and conversely the hybrid cell assignments confirm the assignments made by other methods. In comparing the mouse and human gene maps we have found a surprising degree of homology between various human and mouse autosomal regions, namely: human 1p and mouse 4; human 4 and mouse 5; human 12 and potentially mouse 6; human 15q and mouse 9, human 10 and mouse 10; human 6p and mouse 17; as well as homology of the human and mouse X chromosomes. This informaion will be of interest in sorting out the evolution of the mammalian chromosome map. In addition, it suggests regions of homology that should be useful in correlating mouse and human diseases. For example, our work has shown there are 4 genes homologous between mouse chromosome 4 and human chromosome arm 1p. In addition, there are many mouse genes including genes for diabetes, and developmental mutants and a gene restricting type C virus replication that map in this area. This suggests that human equivalents for these genes should be found on human chromosome arm 1p by a process of homology mapping. We plan to continue to map mouse genes using these hybrids, and feel this work is a major contribution to mouse genetics.

These hybrids and the rodent x human hybrids segregating human chromosomes (see section 3.12) also provide data on non-random segregation of chromosomes. The most striking examples of this to date, are the absolute exclusion of mouse chromosome 11, and the retention, often in multiple copies of mouse chromosome 15 in the Chinese hamster x mouse hybrids.

3.14 Mapping of Rabbit Genes Using Somatic Cell Hybrids (Moss, Minna).

Somatic cell hybrids of Chinese hamster x rabbit which segregate rabbit chromosomes have been produced and are being studied to determine linkage patterns of approximately 30 different isozyme markers which have been found to be separable between the two species using starch gel electrophoresis. To date we have found that mannose phosphate isomerase and pyruvate kinase-3, which are known to be tightly linked in man and mouse, are also linked in the rabbit. Also, early data suggests that four X-chromosome markers which have been studied also appear linked in the rabbit as they are in man, but with a segregation pattern which implies a major inversion on the long arm of that chromosome. Thus, we are finding evidence of conservation of synteny groups in the rabbit.

3.15 Mapping of Chinese Hamster Genes Using Somatic Cell Hybrids. (Minna in collaboration with W. McBride)

Hybrids formed between clonal lines of continuous mouse cells and normal cells taken directly from the Chinese hamster segregate Chinese hamster chromosomes and thus can be used to map Chinese hamster genes. We have been studying the segregation of a large number of Chinese hamster isozymes in these hybrids. We are again looking for conservation of autosomal synteny groups. This work will continue.

3.16 Computer Programming for Gene Mapping and Cell Culture Work. (Minna and Moss).

In the past year a system for the storage and retrieval of data for the cell culture laboratory has been developed. Because information on several thousand somatic cell hybrids and their subclones must be kept, it was found unfeasible to continue manual record files in which extensive cross-indexed searches of the data are impossible. Utilizing a telephone-accessed time-shared system based at NIH, we have currently in operation a system for the storage of inventory files and our cell lines. In addition the capability to analyze the data for mammalian gene mapping experiments exists on the current system. In the next year we plan to extensively broaden the scope of the laboratory data system by replacing the time-shared system with our own PDP-11 minicomputer, which will enable us to continue the aforementioned information storage and processing, and in addition allow for real time data acquisition with such devices as the flow microfluorimeter and gamma counter.

3.17 Influence of Primate Tropic Type C Virus on Tumorigenicity of Human Lung Cancer Cells. (Carney, Gazdar).

In vitro infection of human cells with non-transforming type C viruses does not alter cell doubling times or cloning efficiency. Previous rodent cell experiments have demonstrated that chronically infected homologous tumor cells are less tumorigenic than non-infected control cells, presumably as a result of altered immunogenicity. Human tumors passaged in athymic nude mice are frequently contaminated with murine xenotropic virus. We have performed experiments to determine whether virus infected heterotransplanted cells are less tumorigenic in nude mice than uninfected cells. The human lung adenocarcinoma cell A549 was chronically infected with baboon endogenous virus, M7. Preliminary results indicate that virus infected A549 cells are less tumorigenic than uninfected cells.

3.18 Production and Characterization of Monoclonal Antibodies Using Somatic Cell Hybrids (hybridomas). (Minna, Moss, Huberman).

In the past 2 years several investigators have been able to produce antibody of predetermined specificity by using the techniques of somatic cell hybridzation. Mice are immunized against the desired antigens, their spleens are removed and the cells fused to clonal lines of mouse myeloma cells using polyethylene glycol (PEG) and hybrid clones isolated in selective media. Some of these clones are found to make, in addition to the paraprotein of the myeloma cell, a monoclonal antibody against the immunizing antigen. The amount of antibody made is large, and this technique promises to revolutionize immunology, serology, and the usefulness of immunological reagents for biochemical purification. A major laboratory effort thus has been started on this technique. A variety of parental myeloma cell lines with selectable markers have been obtained, recloned and screened for their ability to generate hybrids. The PEG fusion technique, and selection of hybridomas in microwells has been developed. Because many thousands of clones need to be screened, techniques for replicate plating, and handling of the large number of specimens are being developed. Radioimmunoassays, radiobinding assays, and hemaglutination assays, have been developed as a sensitive large scale screen for antibody production. In addition, the growth requirements of the hybridomas appear to be novel (many are fastidious) and these are being worked out. Hybridomas have been injected into mice, to use this *in vivo* system to grow large amounts of tumor, and harvest large amounts of antibody from ascites or mouse serum for preparative purposes.

3.181 Production and Characterization of monoclonal antibodies Against Lysozyme. (Huberman, Potter, Minna).

In collaboration with the laboratory of Dr. Michael Potter we have been making monoclonal antibodies against egg white lysozyme. The genetics of the mouse response to lysozyme has been worked out by other labs, as have the sequence, and x-ray crystallography. Thus, this is a well defined protein. Hybridoma culture fluids antibodies have been identified which bind to lysozyme and these cells are being recloned. Dr. Potter's lab plans to sequence these antibodies, compare their variable regions, and attempt crystallization of the antibodies for three dimensional structural studies. We plan to continue this work on this model system.

3.182 Production and Characterization of Monoclonal Antibodies Against Staphylococcal Nuclease. (Moss, Sachs, Minna).

In collaboration with the laboratory of Dr. David Sachs we have been making monoclonal antibodies against staphylococcal nuclease. Again the immunogenetics, primary sequence, and three dimensional structure of this protein have been worked out. It is a model system for studying the monoclonal antibodies. We plan to continue this work.

3.183 Production and Characterization of Monoclonal Antibodies Against Mouse Lymphocytes. (Moss, Mathieson, Asofsky , Minna).

In collaboration with the laboratory of Dr. R. Asofosky, and Dr. B. Matheison, we have been making monoclonal antibodies against mouse lymphocytes. In these experiments, mouse x mouse immunization are done that should only stimulate antibodies to Ly 1.1 antigens. These antibodies should be of great use to immunologists in sorting out the biology of the mouse T lymphocyte. We have hybridomas producing antilymphocyte antibodies and we are in the process of cloning and characterizing these lines.

3.184 Production and Characterization of Monoclonal Antibodies Against Human Lung Cancer Cells. (Huberman, Minna)

We have been making monoclonal antibodies against human small cell lung cancer lines. We are immunizing mice with nude mouse transplantable lung cancers, and then testing for binding activity in a radiometric assay to a panel of human cells. We are trying to obtain samples from patients that are still alive to facilitate comparison of antibodies against tumor cells and normal tissues. We have screened over 1000 clones and have found binding activity against human cells in several lines. By testing these against bronchial washings from patients specificity for antigens on small cell lung cancer from other cells in the bronchial washings may be demonstrated. We feel such reagents would be of great clinical usefulness.

3.185 Other Monoclonal Antibodies

Other hybridomas that are in the process of being made involve immunization with human red cells, lymphocytes, and polypetide hormones known to be secreated by small cell lung cancers. In addition, all of the human monoclonal antibodies will be tested on somatic cell hybrids for purposes of mapping the human genes controlling their synthesis.

3.19 Growth Factor and Hormone Requirements of B Cell x B Cell Hybridomas. (Huberman, Moss, Minna).

We have found that many hybridomas have stricter growth requirements than the parental mouse myeloma cells. These probably reflect the requirements of normal B cells in vitro. For example, some hybridomas only will clone on a feeder layer despite the ability of the parent cell to clone under standard growth conditions. Thus, we plan to continue to elucidate the hybridoma growth factor requirements.

3.20 Mouse Myeloma x Normal Human B Cell Hybridomas. (Minna and Broder).

In collaboration with the laboratory of Drs. Broder and Waldman, we have been trying to make hybridomas between mouse myeloma cells and human peripheral blood lymphocytes that have come from individuals boosted against tetanus toxoid. This should be a model system for generating human monoclonal antibodies against defined antigens. These studies are in progress.

3.201 Localization of Mouse Genes Coding for Immunoglobulins Using Somatic Cell Hybrids. (Moss, Minna, Riblett, Weigart).

In collaboration with the laboratory of Drs. M. Weigart, Riblett in Fox Chase, Philadelphia, Penna, we have been studying the localization of genes for mouse immunoglobulins. Their lab has been testing Chinese hamster x mouse hybrid cells segregating mouse chromosomes with molecular probes directed against mouse heavy and light chain genes. The localization of such genes will be of evolutionary interest and also of importance for monoclonal antibody production. A major problem with the hybridoma technique is the segretation of chromosomes carrying the genes for the new antibody. If the localization of the genes were known, we would be better able to generate selective systems to hold these chromosomes in hybridomas.

3.202 Mouse Myeloma x EBV Transformed Human Lymphocytes (Minna and Zurawski)

We have been trying to generate hybridomas by fusing mouse myeloma cells with cloned human EBV transformed lymphocytes that make anti-tetanus antibody in collaboration with the Cardiology Unit, MGH, (Drs. Zurawski and Haber). The objective is to increase the amount of human antibody produced by the presence of the mouse myeloma secretory and synthetic apparatus.

3.21 Laboratory Studies of Human Tumor Immunology (West).

In collaboration with the laboratory of Immunodiagnosis of the NCI, (Dr. Huberman), an immunology laboratory has been established to investigate various aspects of the immunobiology of malignant disease. It is anticipated that such investigation may facilitate rational design of future trials of immunotherapy involving NCI-VA MOB lung cancer patients and will lend itself to analysis of the immunologic aspects of ongoing or incipient trials with thymosin and autologous bone marrow transplantation. Studies in this laboratory have been initiated in both animal and human systems:

3.211 Murine Studies

Although animal models must be viewed with suspicion regarding their relevance to human tumor immunology, experimental animal systems can provide important insight into various aspects of host-tumor interaction. The poorly differentiated adenocarcinoma of the lung, Madison 109, disseminates widely following subcutaneous injection into BALB/c mice. The reported response of this tumor to the interferon-inducer pyran polymer as well as to the conventional cytotoxic agent adriamycin makes it a model of particular interest for NCI-VA studies. Studies in this system in our laboratory have included: 1) Preparation of purified membrane vesicles as a source of putative tumor-associated antigen (TAA); 2) Detection of the proliferative response to "TAA" in the mixed lymphocyte-tumor cell culture; 3) Detection of the activity of natural killer cells and cytostatic macrophages against this tumor. Investigations to date have revealed: 1) A weak proliferative response to TAA inhibited by rayon adherent (suppressor) cells in the spleens of tumor-bearing animals; 2) Modest but significant susceptibility of Madison 109 to the effects of natural killer cells _in vitro_; 3) Striking susceptibility of this tumor to the effects of activated macrophages _in vitro_. Future studies will focus on chemoimmunotherapy of this tumor using specific and nonspecific active measures and adoptive immunotherapy with both thymosin and _in vitro_ sensitized cells.

3.212 Human Studies

A. Natural Cytotoxicity

Natural killer cells (NK) have been identified as an important anti-tumor effector population both _in vitro_ and _in vivo_ in rodent models. To clarify the significance of NK in humans and to gather information regarding the general immunocompetence of patients with newly-detected small cell lung cancer, patients and normal subjects have been tested for cytotoxic reactivity against the myeloblast K562 (a standard target of NK cells) and against small cell lung cancer lines established by the NCI-VA Human Tumor Cell Biology Laboratory. Since NK activity has been demonstrated to be strongly influenced by interferon, studies have also involved incubation of mononuclear cells with the interferon inducers poly I:C and pyran polymer overnight prior to cytotoxic testing. These studies have revealed a depression of NK reactivity in with small cell lung cancer patients and preliminary data suggest that this depression is reversed by incubation with poly I:C. Both patients and normal subjects have demonstrated selective reactivity against small cell lung cancer lines. The small cell lines appear to be highly sensitive to the effects of natural killer cells.

B. Proliferative Responses to Tumor Associated Antigens (TAA)

Lymphocyte proliferative responses to autologous human tumor cells are of interest not only as evidence for the existence of TAA on human tumors but also for the theoretical insight they provide into T lymphocyte helper-suppressor immunoregulatory circuits. Analysis of the proliferative response to autologous small cell tumor will constitute the major focus in studies of thymosin and other potential immunotherapeutic agents administered to NCI-VA MOB patients. Few patients, however, have presented with small cell tumor deposits suitable for excisional biopsy. Efforts are currently underway to prepare small amounts of purified membrane from tumor tissue obtained by bronchoscopic biopsy.

3.22 Laboratory studies of tumor Oxygenation, Radiation and Chemotherapy Sensitivity (Ihde).

Administration of radiotherapy in hyperbaric oxygen increases the radiosensitivity of certain murine tumors. Our previous studies have revealed that administration of hyperbaric oxygen at sufficient pressures and for sufficient time periods will suppress ^{3}H-TR labelling index and incorporation into DNA in vivo in all tumor systems investigated. In SQ B16 melanoma only, however, hyperbaric oxygen for15 min at 1-3 ATA increased both these indices of tumor proliferation. It was then elected to study the toxicity and antitumor effect of cyclophosphamide in B16 melanoma under conditions which increased and suppressed the labelling index. Preliminary results indicate that toxicity is enhanced with hyperbaric oxygen exposures that reduce the labelling index, but not under conditions that increase it.

3.23 Laboratory Studies on the Kinetics of Human Malignancies (Bunn, Nugent, Brigham).

The effort of this project is directed at determining the cell kinetic properties of human malignant cells in vivo in the patient, in culture, and in the xenograft. By using the information gained from these studies we will try to design better treatment regimens. To this end, we have purchased a Coulter TPS (r) cell sorter and are performing kinetic studies with this instrument. We have shown the majority of myeloma cells and Sezary cells are aneuploid.

Cytogenetic studies are being performed simultaneously by Dr. W. Peng, MB/DCT. We are developing techniques so that myeloma cells are identified and isolated from normal marrow cells by staining with fluorescent antiserum against their paraprotein. We have collaborated with Dr. S. Shackney LCP/DCT and Mr. Pendland DCT in contracting ARTEE Systems, Inc. to develop a microscope based system for providing semi-automated grain counts and DNA contents from autoradiography studies.

Using the above techniques we have shown that the doubling times of small cell lung tumors average 67 days and are much longer than previously reported. The clinical implications of these findings have been published in collaboration with Dr. Shackney. Cytokinetic studies on patients with the Sezary syndrome have shown that the malignant cells do not grow in the skin but rather migrate there from the blood. These findings has not been previously recognized and are submitted for publication.

3.24 Laboratory Studies of Megakaryopoiesis

We have shown that the DNA content of megakaryocytes (4N, 8N, 16N 32N and 64N) can be measured by the TPS cell sorter. Shifts in megakaryocyte ploidy after stimulation with a variety of agents are under investigation. DNA content measurements of megakaryocytes in tissue culture are in progress in collaboration with Dr. R. Levine, Hematology Section, WVAH.

3.25 Laboratory Studies of Tumor Blood Flow (Cohen)

Cancer chemotherapy may be limited by impaired tumor blood flow. Even portions of the periphery of a tumor mass may be poorly perfused while the central regions of a tumor may receive no blood supply at all.

A major factor affecting flow to a tumor is the red blood cell. We have set up assays for studying red cell deformability and filterability and studies of red cells from lung cancer patients in our laboratory indicate significant defects in red cell function both during the pretreatment staging workup and also following intensive chemotherapy. Present studies involve attempts to correct these red cell changes in deformability and filterability.

A bacterial assay system has been developed to measure total blood flow to the tumor in animal tumors or human xenografts in nude mice. The studies indicate considerable stasis within the tumor vascular bed. These stasis changes will inhibit transport of drugs and oxygen to a tumor. The present program in the laboratory involves investigations of vasoactive and other pharmacologic agents in an attempt to improve blood flow.

3.26 Laboratory Studies of the Early Detection of Infection Using Immunoassays (Fossieck, Fedorko).

This laboratory project has continued its work on the development and evaluation of counterimmunoelectrophoresis (CIE) assays for the early detection of infection. Tests for antigens of S. pneumoniae, K. pneumoniae, H. influenzae, N. meningiditis, S. Aureus, C. albicans, and for antibodies against S. aureus, C. albicans, M. pneumoniae, and A. fumigatus have been established. An animal model for the study of endocarditis has been perfected and is in use.

The early detection of infection and the evaluation of fevers of unknown etiology are still of great importance in the management of patients receiving intensive chemotherapy. Counterimmunoelectrophoresis (CIE) (developed in this laboratory in collaboration with Dr. R. Parker, Infectious Disease Section, WVAH) is a technique for rapid diagnosis of infection and was employed in the following studies:

The prospective surveillance by CIE of patients in the NCI-VA Oncology clinical program is continuing. A CIE method fo Aspergillus antibody detection has been developed and has been incorporated into the surveillance program for development of infection along with S. aureus, and C. albicans screening. To date, some 800 initial and sequential studies have been performed on 160 patients. An additional study on these sera is being planned whereby selected samples will be checked for staphylococcal antigen by more sensitive enzyme linked immunosorbent assay (ELISA).

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PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Study and Treatment of Cancer in Children and Young Adults

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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LAB/BRANCH

Pediatric Oncology Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
35	20	15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Acute leukemia, Non-Hodgkin's lymphomas (e.g. Burkitt's), neuroblastoma, rhabdomyosarcoma, osteosarcoma, Ewing's sarcoma and aplastic anemia are studied. In leukemia, we have devised therapeutic regimens which may allow maximum tumor cell kill while minimizing sequellae. Emphasis is on tailoring treatment to individual prognostic variables, and improving the therapeutic ratio in CNS prophylaxis. In the solid tumors, we have developed combined modality approaches to primary disease. In refractory tumors, we are studying the utility of high-dose-therapy and the role of supportive care (platelet and white cell transfusion, laminar-flow protection, autologous bone marrow rescue, total parenteral nutrition) in permitting such therapy. Phase I trials are also conducted in refractory tumors; agents include maytansine, poly I:C/poly-l-lysine, glutaminase, AMSA, and PALA. Aplastic anemia is studied as a model for hematopoietic differentiation. We are exploring the biology of selected tumors, including kinetics, immunology, virology, tumor markers, genetics, biochemistry, and pharmacology. Also studied are the effects of cancer and its treatment on growth, development, and organ function. The psychosocial concomitants of cancer are explored.

PHS-6040
(Rev. 10-76)

Objectives:

1) To devise effective therapeutic regimens in acute leukemia which allow maximum tumor cell kill with drugs and radiotherapy, while leaving intact effective elements of the immune response and minimizing therapeutic sequellae--especially in the CNS.

2) To develop effective combined modality treatment approaches to previously untreated childhood solid tumors, including rhabdomyosarcoma, neuroblastoma, non-Hodgkin's lymphoma, osteogenic sarcoma, and Ewing's sarcoma.

3) To study the possible role of high-dose ("ablative") chemotherapy in advanced solid tumors, and the role of supportive care (platelet and white cell transfusion, laminar-flow protection, bone marrow rescue, hyperalimentation) in permitting such ablative therapy.

4) To study the biology of selected tumors, including kinetics, immunology, virology, prognostic markers, karyotypes, biochemistry, and pharmacology.

5) To assess the pharmacology of selected agents, e.g., high-dose methotrexate, AMSA, maytansine, poly I:C/poly-1-lysine, glutaminase, and PALA in humans.

6) To study the short- and long-term effects of malignant disease and cancer treatment on growth, development, and organ function.

7) To study the psychosocial concomitants of cancer in young patients, their families, and their therapists.

Methods and Major Findings:

A. Therapeutic Regimens for Acute Lymphatic Leukemia (ALL)

1. Protocol 72-1 was designed to compare the utility of maintenance chemotherapy versus maintenance chemotherapy plus immunotherapy. All patients received 5 day remission induction courses of POMP (prednisone, vincristine, methotrexate, 6-MP) until an M1 marrow was achieved. All patients were then treated with systemic consolidation chemotherapy and CNS prophylaxis. The latter treatment consisted of 2400 R to the brain and intrathecal cytosine arabinoside or methotrexate given during irradiation and monthly thereafter for 30 months. Following completion of consolidation chemotherapy and cranial irradiation, patients began a 34 month course of maintenance therapy, divided into 6 treatment blocks. Each block was composed of a 4 month (Phase I) and a 2 month (Phase II) cycle. All patients received identical maintenance therapy during Phase I consisting of 6-MP, methotrexate, vincristine and prednisone. Patients were randomized to receive during Phase II one of three different regimens: 1 of 2 methotrexate schedules, or BCG plus allogeneic cells. A total of 70 patients were entered on this study which was closed to

entry in 1975. Sixty-three patients (90%) achieved complete remission. Of these patients, 29 (46%) successfully completed chemotherapy and have been off of all treatment for up to four years. Twenty-four patients (39%) are in complete remission at 7 years. Five patients relapsed while unmaintained. When one considers the particular set of apparent prognostic variables within our patient population (age, initial white count) these results are similar to or better than those obtained in other major centers during this period of time. It should be noted that 55% of the 72-1 patients had an initial WBC > 25,000/mm^3 and/or were $\leq$ 2 or $\geq$ 10 years old. We found no significant difference in either the relapse rate or the duration of remission between patients treated with maintenance chemotherapy alone or maintenance chemotherapy plus immunotherapy.

2. Protocol 75-1 - Retrospective analysis of protocol 72-1 demonstrated that patients could be divided into good and poor prognostic groups on the basis of certain factors. Poor prognosis was associated with failure to achieve remission following four courses of POMP induction therapy; the presence of meningeal leukemia at the time of the first CSF sampling; age; and initial WBC count exceeding 50,000/mm^3. Of patients in the good prognosis category, 80% remained in remission at 50 months. In the 75-1 study, patients were segregated on the basis of these previously identified prognostic factors. Patients in the good prognosis group were treated conservatively as in the 72-1 protocol. Patients in the poor prognostic group were treated with a much more intensive chemotherapeutic regimen and randomized to receive or not receive immunotherapy during induction and maintenance. Patients randomized to receive immunotherapy were given the methanol-extraction-residue of BCG (MER). Twenty patients with ALL have been entered on the 75-1 protocol, four in the good prognosis group and 16 in the poor prognosis group. (This distribution appears to reflect a trend in the NIH referral pattern). Nineteen of the 20 patients achieved complete remission; seven of the 16 patients in the poor prognosis group have already relapsed, with no significant difference between those receiving MER and those not receiving immunotherapy. All four patients in the good prognosis group remain in complete remission. Projected remission duration and survival data in the poor prognosis group has indicated a relapse rate and remission duration for the poor prognosis patients no different than was observed for the earlier 72-1 poor prognosis patients. Thus, intensification of chemotherapy as practiced in protocol 75-1 does not improve the outlook for poor prognosis ALL patients.

3. Protocol 77-02 - In this study, all patients with ALL receive identical systemic treatment without regard to presumed prognostic variables. Our hope is to learn more about the unique biology of each patient such that specific mechanisms of response or failure can be explicated. This information will then be used to guide further treatment. Additionally, this protocol examines prophylactic treatment of the CNS. As indicated previously, the classic therapy offered during CNS prophylaxis consists of cranial irradiation plus intrathecal chemotherapy. We have noted an unexpectedly high incidence (more than 50%) of important neurologic sequellae consequent upon this treatment, and protocol 77-02 attempts to learn in a controlled study whether systemic chemotherapy constructed with regard to CNS pharmacokinetics will produce as good or better a therapeutic result as intrathecal chemotherapy plus cranial irradiation. Our hope is that the former regimen, while avoiding cranial irradiation, will be associated with fewer significant neurologic sequellae. Thus far, 14 patients have been enrolled in this protocol. Nine have received cranial irradiation plus intrathecal MTX as CNS prophylaxis (standard treatment); five have received CNS prophylaxis in the form of high-dose prolonged intravenous MTX infusions. Only one patient (receiving the standard treatment) has suffered a CNS relapse to date, and two patients have relapsed systemically. It is too early to draw conclusions from this study.

4. Multiple Reinduction Attempts with the Same Drugs - Between 1972 and 1976, 56 patients with ALL in relapse have been treated with a reinduction regimen utilizing L-asparaginase, vincristine, daunomycin, and prednisone (Asp, V, D, P). Patients ranged from 2-1/2 - 30 years with a median of 11; there were 39 males and 17 females. This regimen has been used for the treatment of multiple consecutive relapses in these 56 patients, resulting in a total of 125 treatment courses. Of the 125 treatment courses, 114 (91%) resulted in complete remission. Ninety-one % (51/56) of patients who received their first reinduction course of this regimen attained complete remission. Eighty-nine % (31/35) also achieved complete remission on their second exposure to Asp, V, D, P, and 90% (18/20) had a complete remission on their third exposure to this regimen; 14/14 patients who received this regimen for treatment of 4 or more relapses achieved complete remission. Median remission duration (maintenance therapy varied) was similar for patients who had been reinduced 1, 2 or 3 times with this regimen (9 months). Thus, this reinduction schedule is highly effective, with response rates approximating that for primary induction therapy. Remarkably, there was no significant difference in response rates or remission duration between patients treated for only one or for multiple consecutive relapses. The major toxicity encountered was infection secondary to myelosuppression. Hypersensitivity reactions to L-Asparaginase were rare. These results emphasize that the major challenge in the treatment of patients with relapsed ALL remains the development of effective maintenance therapy.

5. Protocol 77-09 - This protocol is currently employed at the time of first and second relapse. Induction therapy consists of the Asparaginase, V, D, P regimen described in #4, and consolidation is accomplished with cyclophosphamide. CNS reinforcement therapy consists of six weekly courses of intrathecal MTX. Patients are randomized to receive one of two types of maintenance therapy, an aggressive schedule which involves periodic "re-induction type" treatment alternating with conventional cycles of 6-MP and MTX, or the conventional cycles alone. Individuals who relapse are re-induced as above, and crossed over to the other maintenance schedule. Thus, this protocol should yield important information regarding the utility of aggressive versus conventional maintenance. Nine patients have so far been enrolled, and it is too early to draw conclusions.

6. Protocol 74-8 - This protocol is employed for third and fourth relapse, i.e., for patients who have relapsed on 72-1, 75-1, 77-02, or 77-09. Induction is identical to that described in #4 and #5. All patients initially receive monthly POMP maintenance. Should they relapse, they are re-induced with Asp., V, D, P and receive POMP cycle maintenance. Thus, this protocol compares two different schedules of the same drugs with respect to remission duration. Thus far, six patients have been enrolled.

B. Studies on CNS Leukemia

1. Intrathecal Methotrexate vs. Cytosine Arabinoside
On protocol 72-1, patients were randomized to receive intrathecal cytosine arabinoside or methotrexate (MTX) as a component of CNS prophylaxis. One of 32 patients receiving MTX and 3 of 29 receiving cytosine arabinoside have developed meningeal leukemia. While these data suggest that both drugs are equally useful as the intrathecal components of CNS prophylaxis, we found that 2 patients that received cytosine arabinoside became blind. While there is no proof of the association between cytosine and blindness, we believe that MTX remains the treatment of choice.

2. Treatment of overt meningeal leukemia - Protocol 73-6
Neurotoxicity associated with intrathecal methotrexate therapy has been shown to correlate with elevated concentrations of the drug in the cerebrospinal fluid as well as with the total cumulative dosage. In our study 19 patients with meningeal leukemia were randomized to receive courses of intraventricular methotrexate via an Ommaya reservoir consisting of either single injections of 12 mg/sq m/dose or a low-dose "concentration x time" (C x T) schedule of 1 mg every 12 hr for 3 days. There were no significant differences between the two treatment groups in the rate of remission induction, the number of relapses, or the durations of remission. The mean (± 1 SD) cumulative methotrexate dose was 66 ± 41 mg/sq m in the C x T group and 173 ± 64 mg/sq m in the 12 mg/sq m/dose group ($p > 0.005$). Neurologic toxicity occurred in one of the eight patients in the C x T group and

in seven of ten patients in the 12 mg/sq m/dose group (p >0.05). These observations suggest that the C x T dosage schedule is less neurotoxic and equally effective in the treatment of central nervous system leukemia.

3. Intrathecal (IT) versus intraventricular (IV) methotrexate (MTX) in overt meningeal leukemia. We compared IV MTX delivered via the Ommaya reservoir versus IT MTX in the treatment of established meningeal leukemia using each patient as his own control. Ten children who had recurrence of CNS leukemia despite monthly IT MTX were reinduced and maintained with IV MTX. Remission duration with IV MTX was compared with the patient's previous remission duration in association with IT MTX. Seven patients had a longer and one a shorter CNS remission with IV MTX than with IT MTX. Three patients who died of systemic leukemia while remaining in CNS remission on IV MTX had no evidence of CNS leukemia at autopsy. There were three CNS relapses during 4,009 patient days at risk in the IV group in contrast to 10 CNS relapses in 3,312 days at risk in the IT treatment group. These data suggest that IV chemotherapy is more effective against overt CNS leukemia than IT.

4. Overt meningeal leukemia: New studies
   We have extended the duration of the "CxT" approach such that 2 patients have received 5 days of CxT methotrexate via indwelling subcutaneous Ommaya reservoirs. All patients tolerated the regimen well without overt signs of methotrexate neurotoxicity. The prolongation of the CxT approach to five days allows for maintenance of prolonged therapeutic CSF MTX concentrations and thus theoretically exposes more leukemic cells to this phase-specific agent as they pass through S phase. We now plan to compare 5 day CxT versus a single intraventricular MTX dose.

   We are also studying the intravenous approach to the treatment of overt meningeal disease. Recent studies in patients and in a subhuman primate model have suggested that therapeutic levels of MTX can be achieved in the CSF following intravenous injection. Since it is likely that intravenous administration is a more physiologic approach, we have treated 2 individuals with overt meningeal leukemia with high-dose 42 hr intravenous MTX. Both patients had therapeutic concentrations of MTX in the CSF and demonstrated a dramatic decrease in CSF blast counts. The systemic approach to the treatment of meningeal disease merits further exploration.

   We have also studied the CSF pharmacokinetics of aminopterin in a subhuman primate model and find that this drug, because of its slow egress from the CSF, merits study in the intrathecal treatment of overt CNS disease.

5. Sequellae of prophylaxis and treatment of meningeal leukemia: Utility of computer-assisted tomography in detecting MTX leukoencephalopathy after treatment of overt CNS leukemia.
Although MTX has been effective in treating overt meningeal leukemia, its administration can result in significant toxicity, including the recently recognized syndrome of MTX leukoencephalopathy (confusion, forgetfulness, poor mentation, and/or progressive dementia). Histopathological examination of brain tissue from these patients has revealed multiple foci of necrosis, mainly involving the white matter. We studied five children with ALL who had developed encephalopathy following treatment with intrathecal or intraventricular MTX by use of computed tomographic scanning. Utilizing this new diagnostic technique, we were able to define two types of CT scan abnormality in these patients: A symmetrically decreased attenuation coefficient in the centrum ovale of each hemisphere and in the region surrounding the lateral ventricles, and, in some patients, calcification in the basal ganglia. The apparent ability of CT scanning to detect MTX leukencephalopathy suggests that both baseline and follow-up CT scans should be performed in all patients so treated.

6. Abnormal computed tomography scans in children with ALL following central nervous system prophylaxis.
Thirty-two asymptomatic patients with acute lymphocytic leukemia, who had received prophylactic cranial radiation (2400 rads) and either intrathecal methotrexate or cytosine arabinoside were studied by computed tomography of the brain 19 to 67 months after initiation of prophylaxis. Seventeen of 32 (53 per cent) had one or more abnormal findings. Dilation of the ventricles (eight patients) and widening of the subarachnoid spaces (nine patients) were equally distributed among patients in both intrathecal-chemotherapy groups. Areas of decreased attenuation coefficient (hypodense, abnormally radiolucent regions) (four patients) and intracerebral calcification (one patient) -- lesions previously described in methotrexate leukoencephalopathy -- were found only in those who had received intrathecal methotrexate. Mild central nervous-system dysfunction was detected in seven patients but did not correlate with the presence of tomographic abnormalities. Nevertheless, these tomographic findings may represent preclinical lesions. The unexpectedly high prevalence of such abnormalities contrasts with the essentially normal tomographic findings in a control group with acute lymphocytic leukemia who received no central-nervous-system prophylaxis. These reults suggest that alternative approaches to such prophylaxis should be considered.

7. Neurological and Psychological Evaluation of ALL Patients Who Have Received CNS Prophylaxis.
In collaboration with Dr. Bruce Adornato (NINCDS), we have performed a comprehensive neurological evaluation of the 32 apparently asymptomatic patients discussed in #6. Abnormalities of both the peripheral and central nervous system were detected. Peripheral neurological findings (decreased deep tendon reflexes, distal lower extremity

motor weakness, altered vibratory sensation, and paresthesias) were found in 19 patients. These findings are probably secondary to vincristine. In addition, 8/32 patients manifested ataxia; two of these had lateral gaze nystagmus. However, these findings did not correlate with the presence of CT abnormalities, nor with the particular type of intrathecal chemotherapy.

Comprehensive psychometric tests have been administered to these same patients by Drs. Moss and Nannis (NIMH). The results are compared with data from three different control populations -- normal siblings, patients with Ewing's Sarcoma at NIH (not receiving CNS treatment), and children with cystic fibrosis (also subject to long-term hospitalization effects). Follow-up tests are performed 1 year later. Preliminary results indicate deficits among the leukemic patients, particularly in areas of cognitive intellectual function.

A prospective evaluation of the effects of leukemia treatment on psychological function is also underway.

8. Neuro-Endocrine Function in ALL Patients Following CNS Prophylaxis
The same 32 patients described in #6 and #7 are undergoing insulin tolerance testing to assess the status of the hypothalmic-pituitary axis. Of 15 patients thus far tested, 13 have been found to have abnormal growth hormone responses following a hypoglycemia challenge. These results are compared with data from earlier ALL patients who did not receive CNS prophylaxis. The analysis includes correlation with growth velocity.

C. Prognostic Assays in ALL

1. Adenosine deaminase - To facilitate the selection of poor prognosis patients for tailored regimens, it is important that new markers be developed to distinguish functional subcategories of lymphoblasts. In addition to a continuing examination of the role of immunological cell surface markers, we are investigating possible biochemical means of distinguishing lymphoblast subtypes. In this regard, we have found that the levels of adenosine deaminase are significantly elevated in lymphoblasts as compared to the levels in normal lymphocytes. Moreover, we have found a significant correlation between these elevated adenosine deaminase levels and lymphoblasts with "T-cell" markers, whereas adenosine deaminase levels are not increased in "null" cell leukemia. This finding may have diagnostic, prognostic, and therapeutic implications.

2. Glucocorticoid receptors
In view of the known difference in susceptibility to glucocorticoids among functional subcategories of lymphocytes in the mouse, and the awareness that different subcategories of acute lymphocytic leukemia (ALL) may represent proliferation of different clones of lymphoblasts, we examined lymphoblast populations with known surface markers for

differences in specific gluocorticoid receptor (GR) activity. Lymphoblasts were divided into those which formed sheep erythrocyte rosettes ("T" lymphoblasts) and those which lacked all surface markers ("null" lymphoblasts). The latter were further subdivided by their ability to stimulate allogeneic donors in mixed lymphocyte culture (MLC). Lack of stimulation in MLC is a characteristic of "T" cells. Eighteen patients with T lymphoblasts had binding sites ranging from 0 - 5,887 sites/cell (median 2,173) ($p > .001$). Nine patients whose lymphoblasts lacked identifiable surface markers but failed to stimulate in MLC had intermediate values for receptor sites. Thus it appears that "null" lymphoblasts are more likely to have a higher number of GR than "T" lymphoblasts. The differential capacity of lymphoblasts to bind steroid may prove useful in designing therapeutic regimens for the different subcategories of ALL.

D. Treatment of Refractory Acute Leukemia with High-Dose Chemotherapy - A significant number of patients with acute leukemia continue to experience relapse and rapidly develop resistance to conventional chemotherapy. High-dose chemotherapy followed by allogeneic bone marrow transplantation has been attempted in this circumstance and has met with a degree of success. The question arises whether the high-dose chemotherapy alone can induce a significant percentage of remissions. In this study, we employed a high-dose chemotherapy regimen in relapse leukemia patients. The regimen was similar to one previously used as preparation for bone marrow transplantation. Seventeen patients (12 ALL and 5 AML) with one or more previous relapses were treated with "TEAM" (cytoxan 50 mg/kg/day, days 1, 2, and 6; Ara-C, 100 mg/M^2 days 3-6; 6-thioguine, 100 mg/m^2, days 3-6; and methyl CCNU, 150 mg/M^2, day 5). Of the 17 treated patients, only three attained complete remission, the longest lasting for 73 days. The results were disappointing and toxicity was great. Thus, we find little to support the use of this high-dose combination chemotherapy regimen in refractory acute leukemia.

E. Studies on the Treatment of Solid Tumors

1. Utility of High-dose Chemotherapy in Metastatic Neuroblastoma - Protocol 75-8 sought to determine whether very high doses of established agents are useful in the treatment of metastatic neuroblastoma, a disease with a current 2 year survival of less than 5%. The study employed a combination of those agents heretofore proven most effective against neuroblastoma, i.e., vincristine, DTIC, and cyclophosphamide. The cyclophosphamide was administered in progressively higher doses, in the hope that the tumor would demonstrate no plateau in its dose-response curve. Vigorous hematologic and antimicrobial supportive care were employed in tandem with this protocol in order to counter the profound myelosuppressive effects of the escalating cyclophosphamide. Eight patients were enrolled in the protocol. Five patients completed three full cycles of chemotherapy; 2 completed 2 cycles. One

patient, who presented with widespread involvement of his abdomen and pleura, did not have bone marrow involvement at that time. His bone marrow was stored, and he received 2 courses of high dose cyclophosphamide and DTIC. Following the second course, he was given total body irradiation and an autologous marrow reinfusion. He manifested a dramatic anti-tumor response but died of pulmonary radiation toxicity. Although all 8 patients demonstrated partial clinical responses, all died of causes directly or indirectly related to the chemotherapy per se (1 patient died in hepatic failure, presumably chemotherapy-induced; one of radiation toxicity; and the remaining six died of infections which occurred during the period of bone marrow hypoplasia following chemotherapy). Autopsies were performed in 7 of the 8 patients; all had evidence of residual neuroblastoma.

New studies are planned which emphasize agents not previously tested in this disease, e.g., streptozoticin, mitomycin, and L-dopa methylester.

2. Studies on the Treatment of Metastatic Osteogenic Sarcoma
    a. Protocol 75-3 - Eleven patients with metastatic osteogenic sarcoma were treated with cyclophosphamide, adriamycin, and, in some cases, high-dose methotrexate. In the event of metastatic progression, the dose of cyclophosphamide was escalated by increasing the number of consecutive daily infusions (dose, 45 mg/kg/day). The results indicate that metastatic osteogenic sarcoma is marginally responsive to adriamycin and cyclophosphamide but rarely responsive to high-dose methotrexate as administered in this trial. The response of metastatic lesions was not improved by escalating the dose of cyclophosphamide.

    b. Protocol 77-03 - This study employs total surgical debulking of overt metastatic disease (Phase I) followed by intensive sequential treatment with various agents known to be active in this disease (Phase II). Following pulmonary resection, patients are given 1 week of high-dose methotrexate followed by exposure to 3 alkylating agents (L-PAM, DTIC, and cyclophosphamide). During the severe bone marrow hypoplasia which follows such treatment, patients are offered laminar-air-flow isolation, platelet and granulocyte transfusions, and autologous bone marrow rescue where appropriate. Following recovery of peripheral counts, patients are then exposed to 1 week of treatment with adriamycin and cis-platinum. Eighteen months of maintenance with high-dose methotrexate alternating with adriamycin then follow. Patients who cannot be totally debulked at the initiation of this study are treated with high-dose methotrexate (pharmacologic Phase I) in the hope that their lesions will be altered in such a way as to then permit total surgical resection, followed by the intensive sequential chemotherapy described above. To date, we have attempted initial pharmacologic debulking of metastases (Phase I) in 6 patients, and a surgical Phase I in 8. Of the 6 receiving high-dose MTX initially, 4 were given repeated 6 hr infusions and 2 were randomized to repeated 42 hr. infusions. Four of the 6 showed no response; a fifth patient had complete resolution of pulmonary metastases (6 hr. MTX), and the sixth became operable (42 hr. MTX).

Of the 10 patients entering Phase II (intensive chemotherapy in the absence of macroscopic tumor), 5 are free of disease at a median of one year (2-16 months) after Phase II induction. One relapsed at 11 months, 2 relapsed before maintenance MTX was begun, one died of drug toxicity during induction, and one is not yet evaluable. It is significant that the 50% who are free of disease at a median of one year had recurrent metastases at frequent intervals prior to this new approach. Toxicity has been tolerable, peripheral blood counts recovering in < 2 weeks with/without marrow infusion.

c. Protocol 77-08 - Patients who fail 75-3 or 77-03 are given cis-platinum alone, or, if they have never been given adriamycin, are randomized to platinum ± adriamycin. Patients with other relapsed sarcomas may also enter 77-08. Of 17 patients, only 2 partial responses have occurred.

d. Protocol 78-02 - For patients who fail 77-08, 5-azacytidine is given. Results are not yet evaluable.

3. Rhabdomyosarcoma

a. Protocol 76-4 (primary treatment) -
Previous studies have shown that combination chemotherapy has significantly improved the survival of children with localized rhabdomyosarcoma. Initial therapy for these patients has usually included surgical excision. However, the sites of tumor involvement (head and neck, GU) often make such surgery mutilative or functionally impairing. Part A of this protocol is designed to assess the utility of initial chemotherapy in "pharmacologically debulking" the primary tumor so as to provide an alternative to "radical surgery." Patients with lesions judged to be surgically resectable are randomized to either initial chemotherapy (followed by radiation and surgery) or to the standard approach of surgery, followed by chemotherapy and radiotherapy. In contrast to the situation with localized tumors, combination chemotherapy has failed to improve the survival of patients with metastatic rhabdomyosarcoma. For such patients, part B of this protocol addresses the utility of intensive (escalating) combination chemotherapy (in conjunction with hematologic and microbiologic support) versus conventional dose combination chemotherapy. Nine patients have entered this protocol (Part A or B) to date. Three patients presented with potentially resectable, localized disease; two were randomized to the "initial surgery" arm of Part A and

one to initial chemotherapy. Six patients presented with resectable or metastatic disease. Three patients with metastases were randomized (Part B) to receive combination chemotherapy with conventional doses of vincristine, actinomycin, adriamycin and cyclophosphamide. Radiation therapy was also administered to primary and metastatic sites. All achieved complete remission, but two patients relapsed six months later. The fourth Part B patient with metastases was randomized to the "intensive chemotherapy" arm, and although this patient's tumor responded poorly to the initial lower doses of chemotherapy, it showed an improved response to escalated doses of chemotherapy. However, toxicity was severe and the patient died with cyclophosphamide-induced hemorrhagic myocarditis. The fifth Part B patient presented with an unresectable rhabdomyosarcoma. Although a partial response was obtained with chemotherapy and radiation, the patient's disease recurred and was then unresponsive to chemotherapy. The sixth Part B patient had a complete remission and remains free of disease.

b. Protocol 75-5 (relapse treatment)
Eight relapsed patients have entered this protocol which employs escalating cycles of intensive combination chemotherapy. Four patients completed the protocol, one attaining a complete remission, and three others partial remission or stabilization. All four patients, however, subsequently relapsed. Four other patients died while on study. Two of these patients had shown significant tumor regression but experienced fatal toxicity (systemic candidiasis and radiation-induced pericarditis). The remaining two patients showed initial tumor stabilization but later died of progressive disease.

c. Diagnostic Studies -
Pathological specimens from patients with undifferentiated sarcoma or rhabdomyosarcoma often present a diagnostic dilemma. To clarify this situation, electron microscopy and immunofluorescent labelling with antimuscle antibody are being utilized. In addition, tumor specimens from two patients have been placed into tissue culture and should provide a useful resource for studies on differentiation, kinetics, and metabolism. Previous studies have shown that tumors from patients with rhabdomyosarcoma have abnormalities in myosin banding; these studies are continuing, a major objective being the elucidation of potential tumor markers. Selected patients are studied with radio-autography and flow-microfluorimetry to assess the kinetics of this tumor.

4. Studies on the Treatment of Non-Hodgkin's Lymphoma

a. Primary treatment (75-6)
Clinical studies in Burkitt's lymphoma have been directed towards study of combination therapy (COMP) consisting of cyclophosphamide,

vincristine, methotrexate and prednisone. Two courses of this therapy are given followed by two doses of cyclophosphamide (each 45 mg/kg) and abdominal irradiation to patients with abdominal tumor. Twenty-two patients have been entered into this study. All except 2 had abdominal tumor and in 3 all abdominal tumor was resected. In June 1977, protocol 75-6 was closed to patient admission. Recent review of this data has indicated that the overall relapse rate of the 22 patients with Burkitt's lymphoma is close to 50% - identical to that of the previous protocol 74-0 (COM). Because four patients who relapsed achieved sustained remission following high-dose therapy (BACT) or therapy for isolated CNS relapse, the overall survival rate is 77%.

b. New Primary protocol (77-04)

Since July 1977, a protocol consisting of alternating cycles of cylcophosphamide, vincristine, adriamycin, prednisone, and high-dose methotrexate (given as a 42 hour infusion with leucovorin rescue) has been initiated for all patients with non-Hodgkin's lymphoma (including Burkitt's). The objectives of this protocol are:

1. To provide a standard therapy for all patients with non-Hodgkin's (diffuse) lymphoma such that factors which may affect prognosis may be discovered, e.g., histological type, surface markers, pre-treatment S-phase fraction and treatment-induced changes in the DNA content histogram, and specific disease sites.

2. To derive tumor cell lines from patients with lymphoma in order to study further the biology of the original tumor, e.g., with regard to the possible significance of surface markers and the potential role of EBV in the genesis or behavior of EBV-associated lymphomas.

3. To provide preliminary information as to whether the therapeutic approach - frequent (10 day) pulses of effective drugs - is likely to provide an effective regimen.

Fifteen patients have been admitted to the protocol, 8 Burkitt's lymphoma, 4 undifferentiated lymphoma, and 3 poorly differentiated lymphoma. All patients rapidly achieved complete remission. There have been three relapses so far - two isolated CNS relapses and one isolated testicular relapse. Interpretation of these results would be premature.

c. High-Dose therapy of relapse-
Eighteen patients with Burkitt's lymphoma resistant to conventional therapy have been treated with high-dose therapy consisting of BCNU, cyclophosphamide (1600 mg/M^2 x 4), cytosine arabinoside, and 6-thioguanine. Nine patients received autologous marrow transfusions and 9 did not. Marrow transfusions were associated with a more rapid recovery in the white cell count, but no difference in the risk of serious infection could be discerned in these patients. Four patients (22%) have achieved sustained unmaintained remissions of 6, 20, 30, and 40 months. One of these patients did not receive autologous marrow, the latter 3 did. Four other patients treated with this regimen with histiocytic lymphoma or PDL had transient responses. These data have persuaded us to pursue the approach of high-dose therapy with autografting; attempts to improve on the current results will be made by utilizing total body irradiation in addition to chemotherapy. In view of the major toxicities of these kinds of protocols, they are reserved for patients resistant to conventional therapy.

d. Successful Engraftment of Cryopreserved Autologous Bone Marrow in Patients with Malignant Lymphoma -
In an effort to evaluate the possible utility of cryopreserved autologous bone marrow infusions in man, 19 patients with malignant lymphoma resistant to conventional chemotherapy were treated consecutively with high-dose chemotherapy followed in the first 12 patients by an infusion of their cryopreserved autologous bone marrow; the next 7 patients received chemotherapy alone and serve as controls. Following chemotherapy, severe leukopenia (<100 leukocytes/mm^3) lasted 6-10 (median = 9) days in controls ($p<.001$). Recovery to 1000 leukocytes/mm^3 occurred 10-18 (median = 13) days after chemotherapy in autograft recipients but was delayed until 12-38 (median = 23) days after chemotherapy in controls ($p<.001$). Autografted patients also recovered granulocyte and platelet function significantly faster and had significantly fewer febrile days after chemotherapy than did controls. Cryopreserved autologous bone marrow infusions can hasten hemopoietic recovery in man after high-dose chemotherapy and this earlier reconstitution may be of clinical benefit to the patient.

5. Studies on the Treatment of Ewing's Sarcoma

a. Background -
In October of 1976, the patients on the Ewing's sarcoma study were transferred from the Radiation Oncology Branch to the Pediatric Oncology Branch. A total of approximately 117 patients had been entered on the Radiation Oncology Branch studies beginning in 1964 until the transition time. Of these, approximately 58 remained as active patients. In addition to radiation of the primary

tumor, the patients had been treated with a variety of adjuvant chemotherapy regimens beginning with cyclophosphamide alone in 1964, vincristine and cyclophosphamide from 1968 through 1969, and with cyclophosphamide, vincristine and actinomycin D from 1969 through 1971. In 1971, the final protocol of the Radiation Oncology Branch was begun, consisting of radiation to the primary lesion followed by combination chemotherapy with vincristine, cyclophosphamide and adriamycin as well as CNS prophylaxis (2000 rads of whole brain irradiation and a single dose of intrathecal methotrexate). In mid-1974, a randomized trial of immunotherapy utilizing BCG was added to the current radiation-chemotherapy study, in conjunction with serial immunological evaluation of the patients using both in vitro and in vivo techniques. Immunological findings in these patients, particularly those obtained prior to the initiation of therapy, have been of considerable interest. The clinical results, however, are not significantly different from those obtained without the use of immunotherapy.

We have carefully reanalyzed the data that has been accumulated on Ewing's sarcoma in the Radiation Oncology Branch since the inception of the initial Ewing's sarcoma protocol in 1964 (in collaboration with R. Makuch, Biostatistics Branch). The purpose of the analysis was to verify and update the results previously reported for the treatment schemes that had been used. Patients were therefore segregated by treatment protocol. Patients treated prior to 1969 (that is, treated with cyclophosphamide alone or vincristine and cyclophosphamide) were considered as a single group. Patients treated with cyclophosphamide, vincristine and Actinomycin D were considered as a separate group, and patients treated on the most recent Radiation Branch protocol (vincristine, cyclophosphamide and adriamycin) were considered as a third group. A search was made for prognostic factors within the overall population and within each treatment group. Site of primary disease, serum LDH level at presentation, and metastatic status at presentation were found to be prognostic factors. Attention was then restricted to patients treated in an adjuvant setting, i.e., patients who presented without evidence of metastatic disease. Therapeutic results improved with successive chemotherapy regimens; however, this improvement was of borderline statistical significance ($p = .06$). It also became clear that later treatment groups differed in composition with regard to the known prognostic factors (site of primary disease, serum LDH) when compared to earlier treatment groups. A higher fraction of patients with more favorable prognostic indicators received the later regimens, and therfore would be expected to do better. When the overall results were corrected for these discrepancies in composition, the later treatment groups had results comparable to those of prior treatment groups. In summary, no statistically significant differences were apparent among any of the chemotherapy regimens when treatment groups were corrected for differences with regard to prognostic indicators.

The results with metastatic disease in all treatment regimens remained dismal, with the longest interval of disease-free status for any patient presenting with metastatic disease being 70 weeks. The median survival was 22 months, and the long-term survival rate has been essentially nil.

b. New Studies -

With the background outlined above and with the availability of supportive care facilities in the Pediatric Onoclogy Branch, a series of studies have been initiated to evaluate the efficacy of intensive multimodality therapy in this disease. Patients presenting with metastatic disease are treated with high-dose radiation therapy (5000 rads) to the primary lesion and to known metastatic sites. Patients are also exposed to known effective chemotherapeutic agents and to total body irradiation, the efficacy of which was suggested in studies published approximately 10 years ago (TBI was not used then in conjunction with chemotherapy). Because of the high-dose chemotherapy employed, together with total body irradiation, patients who had no disease detectable in the marrow at the time of presentation have their marrow stored and reinfused subsequent to the high-dose chemotherapy. Immune competence as well as the kinetics of marrow reconstitution are being studied in these patients.

Patients who present with central axis lesions are treated with a regimen similar to that being used for patients presenting with metastatic disease. Patients with distal lesions and without metastases are treated with radiation therapy to the primary lesion as well as vincristine, cytoxan, actinomycin D and adriamycin. They also receive prophylactic pulmonary irradiation based on preliminary data from the Intergroup Ewing's Study and the known efficacy of 1500 to 2000 rads whole-lung irradiation in controlling the common complication of pulmonary metastases. Patients who have recurrent disease and who have not been previously exposed to high-dose chemotherapy are now being given such treatment. Patients who have previously received high-dose chemotherapy go on to Phase II studies. Since October of 1976, approximately 20 new patients have been entered on these protocols, divided almost evenly between the high and low-risk protocols. It is too early to comment on the overall results; however, it is of note that patients treated with the present protocols appear thus far to have a significantly longer disease-free interval than reported with previous chemotherapeutic regimens.

F. Phase I-II Trial of Stabilized Polyriboinosinic Acid.Polyribocytidylic Acid [Poly(I).Poly(C)] in Leukemia and Solid Tumors - Because viruses may be implicated in the etiology of human cancer, we conducted a trial of a new antiviral agent, poly(I).poly(C) stabilized with poly-l-lysine and carboxymethylcellulose [poly(ICLC)]. Unlike the parent compound which is rapidly hydrolyzed by primate serum, the stable derivative induces high levels of serum interferon (IF) and is effective in acute viral infections of subhuman primates. Poly(ICLC) was given intravenously (iv) in 15 daily doses of 0.5 - 27 mg/M^2 to 19 patients with various solid tumors and 6 with acute leukemia (ages 1-65). At least 3 complete trials were conducted at each of 5 dose levels. Toxic reactions included fever (in 100% of trials), nausea (44%), hypotension (28%), thrombocytopenia and leukopenia (68%), erythema (12%), and polyarthralgia plus myalgia (16%). Hypotension and arthralgia-myalgia related to dose level and/or magnitude of IF induction but other toxic manifestations did not. Poly(ICLC) induced significant serum IF levels in 76% of trials and the correlation between dose and peak IF titer was linear. The maximum tolerated dose for all patients at a given drug dose was 12 mg/M^2; at this dose the mean peak IF titer was 5000 reference (ref.) units/ml. At 18 mg/M^2, the peak IF titer was 16,000 ref. unit/ml but severe mylagia-arthralgia was intolerable in at least half the patients and most had significant hypotension. At 27 mg/M^2, 1 patient had acute renal failure. At high doses, iv poly(ICLC) also induced IF in the cerebrospinal fluid. No patients had an objective tumor response to this compound, but our observation that poly(ICLC) at a tolerable dose is the first consistant inducer of high serum IF levels in man suggests that it should be assessed in such human viral infections as the hepatitis B carrier state.

G. Miscellaneous Phase I Studies

1. Maytansine, completed (see report of Clin. Pharm. Branch, NCI); phase II begun.

2. AMSA (77-12): We have entered five patients in this trial (in collaboration with the Medicine Branch). All had ALL or AUL; at a dose of 10-90 mg/M^2 there were no responses, and no overt toxicities.

3. PALA (78-01): One patient (AUL) entered to date (350 mg/M^2); no response, no definite toxicity.

H. Total Parenteral Nutrition Study - Patients with metastatic rhabdomyosarcoma, neuroblastoma, Ewing's sarcoma, or osteosarcoma who are receiving intensive combination chemotherapy are randomized to receive (or not receive) intravenous hyperalimentation. Eight patients have been entered on this protocol, four in each arm. Careful metabolic and immunologic studies are being carried out in all patients. It is notable that even though the patients have been granulocytopenic (PMN < 500/mm^3) from 12 to 21 days, no catheter-related infections have occurred in the patients undergoing hyperalimentation. The relationship of nutritional integrity to host defenses, chemotherapeutic responsiveness, and drug toxicity may be of great importance in patients receiving very intensive drug regimens. This study should provide valuable insights into the metabolic changes which accompany intensive chemotherapy, as well as the utility of intravenous hyperalimentation in their prevention.

I. Psycho-social Concomitants of Cancer

We have previously reported on the families of patients who have life-threatening illness and on physicians who care for such patients. In particular, we explored the factors in the doctor-patient relation which promote anxiety in the doctor to the end that he is less effective than he might be. During the past year, our annual seminar for Clinical Associates on the Doctor-Patient Relation has continued with Dr. Kenneth Artiss as the seminar leader. The seminar continues to be highly successful and has proven to be a model for many other institutions throughout the country, including cancer centers and general hospitals. We have also initiated therapy groups for ill adolescents; these groups are conducted by oncologists and social workers who have received appropriate training in the principles of social and preventive psychiatry. We have also continued a collaborative research effort with the Laboratory of Developmental Psychology, NIMH (M. Yarrow, Chief). The objective of this research is to analyze developmental stages in young patients who have chronic and/or life-threatening disease, to learn the factors which promote normal or abnormal development, and the manipulations which may be of value in dealing with developmental abnormalities. This collaboration has been strengthened during the past year by the addition of several new post-doctoral fellows in Dr. Yarrow's laboratory. We are continuing studies on the nature of communication between staff, family and patient; the effects of isolation within the laminar-flow room as a function of the age of the patient; and the effects of mentation in patients who have received prophylactic irradiation of the central nervous system (as noted elsewhere, we have detected a significant drop in I.Q. among patients so treated).

J. Educational Programs -

Our extensive seminar, lecture and rounding schedule for associates and post-doctoral fellows has been continued. Two weekly Branch seminars emphasize disease entities in hematology-oncology, and research in basic sciences related to cancer. These seminars now attract a large audience from throughout the Institutes. The medical student elective program continues to be successful, with a significant proportion of students applying for eventual Clinical Associate positions.

Significance to Biomedical Research and the Program of the Institute:

Significant progress has been made in delineating the cell of origin in acute leukemia and in identifying factors which may be useful in predicting response to therapy. By dividing the acute leukemias viv-á-vis these prognostic factors, we hope to individualize therapy to the end that remission rate and duration will further improve. In this Branch 80% of patients with "good prognosis" ALL are free of disease 7 years after diagnosis and optimum treatment. The study of steroid receptors is being pursued in the hope that the basic mechanism of leukemic cell kill can be elucidated. We have learned that the acute leukemia cell has extraordinary antigenic complexity; the effects of both specific and nonspecific immunotherapy in this disease have been discouraging. However, as we evaluate the effects of therapy on general and tumor-specific immunity, it is hoped that our understanding of mechanisms involved in leukemia and immune surveillance will increase. The central nervous system is a major site for relapse in acute leukemia. Our studies on route and scheduling of CNS chemotherapy promise an effective and relatively nontoxic approach to this important problem. It is further evident that the most important contribution that can now be made to the treatment of "good risk" patients with acute lymphocytic leukemia is to provide effective CNS prophylaxis without the significant toxicity we have detected in association with cranial irradiation (CT scan abnormalities; neurologic, intellectual, and psychologic dysfunction; growth hormone deficits). By exploiting the elegant monkey model for CNS pharmacokinetics developed in this Branch, the very sophisticated monitoring techniques available to us (e.g., C.T. scans), and our experience in clinical pharmacokinetics, we hope to develop an effective CNS prophylaxis to be administered systemically and hopefully without significant sequellae.

We are beginning to make progress in the treatment of advanced solid tumors that have been refractory to conventional doses of combination chemotherapy. The response in American Burkitt's lymphoma has been particularly gratifying in that a small number of patients with this disease have been treated with high-dose chemotherapy and have had striking long-term remissions at a point in time when the disease has historically been invariably fatal. The role of autologous marrow rescue in this setting, while tentative, also appears to hold much promise. We further believe that the concept of high-dose combination chemotherapy together with maximal supportive care will be useful in metastatic osteogenic sarcoma, in rhabdomyosarcoma, and in Ewing's sarcoma, although our evidence is disappointing in the case of neuroblastoma. In all of these situations, our objective is to debulk the tumor to the maximum extent and then use intensive chemotherapy--prompted in part by the mathematical reasoning of Norton and Simon that the low growth rate of a small tumor volume mandates a large drug dose. In each of the solid tumors we are exploring other interesting observations: the synergistic possibilities of treatment with cis-platinum and adriamycin in the case of metastatic osteogenic sarcoma, pharmacological debulking in the case of localized rhabdomyosarcoma, and the suggestive efficacy of total body irradiation and prophylactic pulmonary irradiation in the case of Ewing's sarcoma.

We have made considerable progress in the development of multi-resource approaches to the psycho-social concomitants of cancer in patients, their families, and therapists. We believe that his approach is exceedingly important, for failure to deal with these concomitants may be reflected in inadequate treatment of cancer. We believe that the Pediatric Oncology Branch has provided a model for coordinated research and care in the psycho-social concomitants of chronic illness which has proven applicable to many other diseases and institutions.

Finally, it should be noted that certain of our studies relate to clinical concerns that are not restricted to cancer treatment: For example, our Phase II trial of interferon inducers has for the first time demonstrated significant interferon induction with minimal toxicity in man. These data should prove useful in a wide variety of infectious disease problems, e.g., control of the hepatitis B carrier state.

Proposed Course:

All of the studies enumerated previously will be continued. Aggressive protocols have been developed for acute leukemia patients who relapse from primary induction protocols but remain sensitive to extant chemotherapy. Increased emphasis will be given to phase I-II trials, in particular studies of maytansine, poly I-poly C/poly-l-lysine, AMSA, PALA, and glutaminase. We shall continue to explore the pharmacokinetics of methotrexate so as to improve the therapeutic index of this drug as it is used in the treatment and prophylaxis of meningeal leukemia. Experiments on the use of high-dose chemotherapy in refractory solid tumors will be expanded; in these studies, chemotherapy is assessed in a situation whereby maximum supportive care is possible, including autologous bone marrow rescue. Our interest in laminar-flow-room prophylaxis, granulocyte transusions, total parenteral nutrition, and empiric antibiosis will continue pari passu with studies of high-dose chemotherapy. We plan to expand studies in clinical and molecular pharmacology and to utilize the results of such studies in planning further clinical chemotherapy trials. Both our clinical and basic studies in the kinetics of hematopoietic reconstitution and differentiation will be used to guide further experiments on clinical marrow reconstitution, using cryopreserved marrow (or stem cells obtainable from the peripheral blood).

In collaboration with the Laboratory of Developmental Psychology, NIMH, we plan to expand our study of stages in normal and abnormal psychological development as they relate to the setting of chronic illness.

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PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-CM-06820-05 PO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Hematological Support of Patients with Cancer or Aplastic Anemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Deisseroth	Head, Experimental Hematology Section	PO C
OTHER:	F. Appelbaum	Investigator	PO C
	D. Wright	IPA Investigator	Tufts Univ.
	R. Abrams	Clinical Associate	PO C
	J. O'Donnell	Clinical Associate	PO C
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COOPERATING UNITS (if any)
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LAB/BRANCH
Pediatric Oncology Branch

SECTION
Experimental Hematology Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12	7	5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The hematologic support of cancer and aplastic anemia patients is studied. A preclinical canine model has permitted determination of the dose of nucleated cells and CFU_c in marrow or peripheral blood which is required to rescue dogs from the lethal effects of total body irradiation, and is being used to study compounds (e.g., dextran sulfate) which increase the levels of CFU_c in the peripheral blood. The role of autologous reconstitution by marrow or peripheral stem cells is studied in a variety of patients with refractory neoplasms receiving "ablative" chemotherapy. Assays for stem cell reserve in cancer patients under therapy are developed and studied. With respect to granulocyte transfusion, colchicine has been shown to protect cells collected by filtration leukapheresis from human donors. Retrospective analysis of over 1000 platelet transfusions in aplastic anemia patients has revealed the frequent inadequacy of HLA typing as a compatibility system. The capillary platelet migration inhibition assay has been shown to be a useful adjunct to HLA in selection of compatible donors for highly alloimmunized recipients. A clinical trial to define which cancer patients require HLA-matched platelet transfusions for optimal support is underway.

Objectives:

1. To attempt autologous reconstitution of hematopoietic cells following intensive chemotherapy for advanced malignancy, using stem cells collected from marrow or peripheral blood of man; to improve methods of cryopreservation of such stem cells; to establish the dose of stem cells (measured indirectly by the CFU$_c$ assay) and precursor cells (committed to myeloid and erythroid differentiation) necessary for marrow reconstitution following intensive therapy; to collect numbers of stem cells from peripheral blood which are adequate for autologous reconstitution; to study the biology of animal and human hematopoietic reconstitution.

2. To utilize the canine model to identify and characterize compounds (e.g., dextran sulfate) which are capable of expanding the circulating pool of committed hematopoietic stem cells; to identify, for each chemotherapy protocol, periods during hematopoietic recovery following treatment when expansion of the number of circulating stem cells occurs; to develop means of predicting depletion of hematopoietic stem cell reserves in patients undergoing sequential cycles of intensive therapy, using *in vitro* and *in vivo* testing; to determine if reconstitution by peripheral stem cells affords faster reconstitution of the immune system as well as marrow than is found with marrow reconstitution *per se*.

3. To study means of ensuring the survival of granulocytes during cryopreservation, so that such stored cells might be used for transfusion in man, and to reduce the potential hazard associated with the extensive lysis of granulocytes which occurs during storage of autologous hematopoietic cells to be used for reconstitution; to identify pharmacological means (e.g., colchicine) of protecting granulocytes collected by filtration leukapheresis.

4. To extend the encouraging results of the capillary platelet migration inhibition test and the results of our retrospective analysis of determinants of good post-transfusion increments in alloimmunized recipients; we wish to identify in a clinical trial which cancer patients will profit from the transfusion of HLA-matched platelets as opposed to single-donor unmatched platelet transfusions.

Methods Employed:

In vitro assays are used to identify the numbers of stem cells of erythroid or myeloid origin present in marrow and peripheral blood samples. These assays involve incubation of nucleated hematopoietic cells in semisolid culture media (methyl cellulose or agar). The number of colonies formed in 10-14 days constitutes an indirect measure of the stem cell content. Automated temperature-controlled instruments are used for the cryopreservation of marrow. Aminco continuous-flow centrifuges, Haemonetics discontinuous-flow centrifuges, and the Fenwal filtration leukapheresis device are used for the collection of granulocytes and platelets. This latter component is also collected by conventional non-automated centrifugational methods ("split bag method"). Collection of mononuclear cells from the peripheral blood is conducted by use of the Aminco continuous-flow centrifuge. An intramural laboratory equipped with advanced culture and analytical techniques (cytofluorograph, scintillation spectrometers, ultracentrifuges, laminar flow hoods, phase bright-field and fluorescent microscopes) is supported by contract facilities in which canine experiments, *in vitro* assays of platelet compatibility, and histocompatibility phenotyping are conducted. A donor file of 20,000 individuals (all of whom are typed with respect to the A and B loci of the HLA system) is backed up by an interlocking network of 13 centers for donor procurement. Finally, a serum repository with at least 14,000 entries is maintained for clinical and laboratory investigations. A battery of granulocyte functional assays are available (bactericidal capability, phagocytosis, chemotaxis, histological and biochemical assays for enzymes specific for mature neutrophils, and assays for substances within the specific and non-specific granules of mature myeloid cells).

Major Findings:

A. Hematopoietic reconstitution

1. Identification of the dose of CFU_c in bone marrow and peripheral blood associated with hematopoietic reconstitution - The dose of nucleated cells associated with 100% survival following total body irradiation (TBI) has been determined to be 0.5×10^8/kg in a pre-clinical canine model. Dogs were subjected to 1000 rads of TBI at 9 rads/minute and then supported with fluids and antibiotics alone or together with an infusion of their own cryopreserved bone marrow (removed and stored in 10% dimethylsulfoxide in the vapor phase of liquid nitrogen prior to irradiation). All six irradiated animals treated without bone marrow infusions died within 9-12 days. All ten animals receiving greater than 0.5×10^8 nucleated cells/kg (stored for up to 5 months) survived. The dose of CFU_c in marrow necessary for hematopoietic reconstitution was found in the model described above to range between 2000 and 3000 CFU_c/kg. The same model was used to determine the numbers of peripheral blood nucleated cells and CFU_c which are necessary for hematopoietic reconstitution to occur in 100% of animals following TBI. In 10 animals, this dose was approproximately 2000 CFU_c/kg, using cells collected by continuous-flow centrifugation from the peripheral blood. Studies are continuing in the same model to refine the technique of peripheral blood stem cell reconstitution, and to also learn if the dose of mononuclear cells and CFU_c collected from the peripheral blood after dextran sulfate pretreatment (utilized for expansion of the circulating stem cell pool) is different than that observed in the case of untreated animals. We hope to learn if the stem cells present in marrow and peripheral blood (with and without dextran sulfate pretreatment) are similar

biologically with respect to their ability to support hematopoietic reconstitution following intensive therapy.

2. Comparison of Cellular and Humoral Immune Reactivity in Dogs Following Reconstitution with Marrow or Peripheral Blood Stem Cells -

   We have found that the rate of recovery of immune competence in dogs reconstituted as above (following TBI) was slower with stem cells collected from marrow than from peripheral blood. Eight dogs (reconstituted with marrow or peripheral blood stem cells) have been studied. Lymphocyte reactivity in MLC, lymphocyte blastogenesis, antibody response to sheep red blood cells, and skin graft rejection times are being used to test immune function at 50 and 150 days following infusion of hematopoietic cells. All tests have been standardized, and data collected on blastogenesis in 6 animals (4 in the peripheral blood and 2 in the marrow group) suggest that immune reconstitution is much slower with marrow than with peripheral blood stem cells. This observation will be pursued in a larger number of animals.

3. Study of patients undergoing chemotherapy with respect to levels of circulating stem cells in the peripheral blood -

   An assay for the measurement of committed myeloid precursor cells in the peripheral blood of man has been established using a reliable stimulator (extract of human placenta) which can be frozen and will provide reproducible results over time. This assay has been applied to the study of patients undergoing cyclical chemotherapy for Ewing's sarcoma. An increase in circulating CFU_c was seen in the peripheral blood of one patient three weeks following conventional-dose chemotherapy. A larger number of patients undergoing initial therapy for oat cell carcinoma of the lung are being studied in a similar manner so as to identify the optimal time for collection of stem cells from the peripheral blood. A series of mononuclear cell collections is underway to evaluate the conditions under which CFU_c may be efficiently collected using the Aminco continuous-flow centrifuge. Finally, we are evaluating a variety of methods to determine stem cell reserve, so as to plan therapy schedules which will not produce prolonged and profound hypoplasia (etiocholanolone stimulation, the Brubacher coil test, CFU_c measurement, and image processing, in which the maturity of the cells in the marrow and peripheral blood can be measured). This evaluation will be carried out in Ewing's sarcoma patients.

4. Hematopoietic reconstitution of patients treated for high-risk, relapsed, or metastatic Ewing's sarcoma -

   Eight patients have been treated with an intensive regimen consisting of 150 rads TBI (10 doses of 15 rad over five weeks) and a combination of cytoxan (40 mg/kg x 2), Adriamycin (35 mg/M^2 x 2) and DTIC (250 mg/M^2 x 3), followed by reconstitution with cryopreserved autologous marrow cells. Six patients have been treated with the support of marrow infusions and two without such support. Five of the 6 patients exhibited recovery of white cells ($1000/mm^3$) and granulocytes ($500/mm^3$) at 25-28 days following initiation of the chemotherapy, and 21-24 days following marrow infusion. The two patients not receiving autologous marrow infusions required 45-48 days from the start of chemotherapy to achieve equivalent counts. The sixth patient in the marrow infusion group exhibited a course of recovery identical to that of the non-infusion group; there was however transient evidence for hematopoietic recovery early in his course which was not sustained. This patient may have failed to engraft due to a low dose of infused marrow cells, or due to some other unidentified factor. While it appears that the infusion of marrow in this group accelerated recovery in 5/6 patients by approximately three weeks, greater numbers of patients in both groups are needed to confirm this finding.

5. Scleroderma, Sjögren-like syndrome, and chronic graft-versus-host disease-

Four patients, treated for hematologic disorders with bone-marrow transplants from HLA-identical siblings, spontaneously complained of dry eyes 8 to 12 months after transplantation. Four allograft recipients and two recipients of autologous bone-marrow transplants were evaluated for xerophthalmia and xerostomia. Three allogeneic marrow recipients had evidence of keratoconjunctivitis sicca, and two had decreased parotid gland function. All four allograft recipients had minor salivary gland histopathology identical to that of Sjögren's syndrome. The severity of symptoms and histologic lesions corresponded with the severity of chronic graft-versus-host disease. In addition, one patient developed sclerodermatous skin changes, another had discoid lupus erythermatosus, and two patients had laboratory evidence of cholestasis. None of the patients had autoantibodies but all had hypergammaglobulinemia. In contrast, none of the recipients of autologous bone marrow had clinical, laboratory, or histologic findings resembling Sjögren's syndrome.

B. Granulocyte Transfusion -

1. Collection of granulocytes by filtration leukapheresis (FL) -

Previous studies in vitro and in vivo have revealed the mobilization and exocytosis of specific granules by human neutrophils during FL, as a consequence of the neutrophils' adherence to nylon wool. We have undertaken a study of pharmacological means of preventing exocytosis, which may be a major cause of the irreversible changes in functional capability observed in granulocytes collected by this method. A series of granulocyte collections, using the Fenwal leukopak (adhesion leukapheresis), was carried out in 5 healthy male volunteer donors. Two collections were made from each donor: A control collection, and a collection preceded by treatment of the donor with 2.4 mg of colchicine. Granulocytes collected without colchicine pre-treatment exhibited a 30-50% reduction in bactericidal and chemotactic function as compared with circulating donor granulocytes. Colchicine administration totally prevented these in vitro defects in granulocyte function. Colchicine pre-treatment was not associated with a significant reduction in the absolute yield of total leukocytes. These studies also show that in addition to preventing the impairment of bactericidal function and chemotaxis caused by FL, colchicine pre-treatment prevents changes in the surface charge of FL neutrophils and reduces the level of lysozyme detectable in the effluent from the filtration leukopak. These findings suggest that pharmacological means for the protection of granulocytes collected by this method are feasible. Moreover, trials in the preclinical canine model using FL transfusions indicate that our ability to protect dogs from Pseudomonas septicemia after cytoxan-induced hyopoplasia can be improved by pharmacologic manipulation of donor animals.

2. Studies of granulocyte transfusion therapy in the pre-clinical model -

A canine model was devised to evaluate the quantitative aspects of granulocyte transfusion. Beagle dogs were given cytoxan (40 mg/kg) as a single bolus, which rendered them neutropenic within 5 days. All animals were supported with ampicillin, gentamicin, clysis, and platelet infusions. Pseudomonas (1 x 10^8/kg) was infused on the fifth day following cytoxan, and dogs were then assigned to a control group or to receive daily granulocyte transfusions (in addition to fluids and antibiotics). The dose of granulocytes (as collected by the continuous-flow centrifuge) needed to protect neutropenic dogs challenged with a lethal dose of Pseudomonas, was found to be 2 x 10^8/kg. Two and one-half times as many granulocytes were needed to protect dogs when the granulocytes were collected by FL. The increased dose of granulocytes (as collected by FL) needed for protection appears to be due to changes in chemotaxis and bactericidal function known to attend the adhesion of granulocytes to nylon filters. These studies have been extended to show that pre-treatment of the recipients of FL granulocytes with hydrocortisone (25 mg) does not alter the dose of cells needed to provide protection. This result suggests that the use of steroids to avert the side-effects of FL granulocytes does not impair the ability of FL cells to protect recipients. Pre-treatment of canine donors with colchicine is underway to learn if the dose of FL cells needed for protection will be reduced as compared with the requisite dose of untreated donor granulocytes collected by the continuous flow method.

C. Platelet Transfusion

1. In vitro correlates of successful platelet transfusion in alloimmunized recipients -

Over 1000 platelet transfusions have been analyzed in which post-transfusion increments and the HLA phenotypes of the donor and recipient were known. Particular emphasis was placed on transfusions to patients with stable but severe aplastic anemia. The effectiveness of transfusions from a single donor to the same recipient was followed over time to determine whether decreasing post-transfusion increments would occur when there was at least a single HLA antigen mismatch in the donor-recipient pair. In our series, a pattern of decreasing post-transfusion increments consistent with sensitization was seen when a donor-recipient pair was mismatched at the HLA locus W21. Possible sensitization patterns were seen with single antigen mismatches involving the loci W33, 29 and 13, but the number of transfusions was too small for these studies to be conclusive. No definite patterns of sensitization were seen when donor-recipent pairs were mismatched at W23, W24, W32, W22, or W12/35. When platelets from the donors were tested for the presence of these specificities, the appropriate antigens (or antigens cross-reacting with antisera presumed to be specific for these antigens) were found. Excellent post-transfusion increments were seen in 25% of recipients when donor platelets exhibited no HLA specificities not seen on the recipient cells. Moreover, 17% of recipients had excellent post-transfusion increments when donor platelets contained one or two antigens not present on the cells of the recipient.

To learn if platelets contained specificities not detected by the HLA system, but which are important in determining post-transfusion increments in alloimmunized recipients, we assessed the ability of the capillary platelet migration inhibition test (PMI) to predict post-transfusion increments in HLA matched or unmatched donor-recipient pairs. The sera from recipients was tested against the platelets of donors which had been documented to give good or poor post-transfusion increments. The PMI was predictive of post-transfusion increments in unmatched but not matched donor-recipient pairs. These data suggest that platelet-specific antigens, as well as HLA antigens, may play a role in the outcome of platelet transfusions in alloimmunized recipients. A clinical protocol has been instituted to assess what categories of cancer patients would benefit from HLA-matched platelet transfusions; patients are randomized to receive matched or unmatched platelet transfusions from the beginning of their therapy. These studies will not only identify the appropriate clinical setting among oncology patients for the use of HLA-matched platelet transfusions, but should also demonstrate if matched support from the start can retard the emergence of alloimmunization to matched or unmatched donor platelets.

Significance to Biomedical Research and the Program of the Institute:

Hematologic support is the cornerstone upon which long-range, intensive therapy of patients with malignancy or aplastic anemia is based. The advances we have made in autologous marrow infusion may permit the use of new and more intensive drug regimens for the treatment of resistant tumors. These studies suggest that human marrow may be cryopreserved and infused safely after prolonged storage, and that its use may be associated with accelerated hematopoietic recovery. Studies conducted both in dogs and man during the past year have also established the feasibility of collecting adequate numbers of stem cells from peripheral blood for hematopoietic reconstitution. By extrapolation of the doses established in the dog model, one can predict that three collections with the Aminco celltrifuge will be adequate for peripheral blood reconstitution in man. This work has provided the information necessary to use peripheral blood stem cells for human hematopoietic reconstitution, and may clarify what advantage this reconstitution may have for the recovery of the immune system following intensive therapy.

Our studies of granulocyte transfusion have been based on a pre-clinical model in which many of the important issues pertaining to the use of this blood component may be examined. Colchicine pre-treatment appears to improve the function of granulocytes collected by adhesion leukopheresis, and this observation may suggest further methods of protection of granulocytes from the effects of nylon filters. Recipient pretreatment with steroid further to reduce the potential for harmful side-effects from the infusion of FL granulocytes may be employed with the knowledge that in the canine model, this maneuver does not impair the ability of the granulocytes to protect the recipient from Pseudomonas septicemia.

Our studies on the role of HLA antigens in platelet transfusion are significant, for platelets are by far the most frequently needed component for the support of cancer patients and the cost of providing HLA-matched platelets to every patient is enormous. Hopefully, our studies will help us identify ways in which to make this important system function more efficiently at a lower cost. In this regard, the platelet migration inhibition assay which we have developed constitutes a simple means of screening donors for platelet transfusions to alloimmunized recipients. Finally, a protocol designed to clarify what clinical settings require the use of HLA-matched platelet transfusions should provide the information to permit the use of this support technique in the most cost-effective manner.

Proposed Course:

Emphasis will be placed on stem cell reconstitution using autologous peripheral blood cells to determine, in carefully designed clinical trials, whether these cells match marrow cells in the acceleration of hematopoietic recovery following intensive chemotherapy. Immunological studies in the pre-clinical canine model reconstituted with marrow or peripheral blood stem cells may help further to define the use of peripheral blood stem cells in man. Hematopoietic reconstitution will continue to be used for the support of patients with non-Hodgkin's lymphoma, Ewing's sarcoma, and neuroblastoma who are undergoing intensive therapy, and may be applied to the support of patients with oat cell carcinoma of the lung under intensive treatment (in collaboration with the NCI-VA Medical Oncology Branch). We shall amplify studies to assess the optimal time following cyclical chemotherapy to collect stem cells from the peripheral blood in patients with Ewing's sarcoma or oat cell carcinoma of the lung. The pre-clinical canine model will be employed to study pharmacological means of amplifying circulating levels of committed myeloid precursor cells, as well as to clarify what moiety of the dextran sulfate molecule amplifies the stem cell pool in the peripheral blood (and through which mechanism).

The studies designed to identify methods of predicting decreased marrow reserves in cancer patients hopefully will contribute to identifying patients at risk for prolonged hypoplasia following chemotherapy, and these results may permit more efficient use of the autologous reconstitution technique. Development of methods of cryopreservation which ensure survival of those granulocytes which, when lysed, contaminate preparations of autologous stem cells should improve the safety of this support technique.

Finally, the proposed and ongoing studies with respect to granulocyte and platelet transfusion may serve to improve the quality of these established methods while increasing their cost-effectiveness.

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23. Wright, D.G., Ungerleider, R.S., Gallin, J.I., and Deisseroth, A.B.: Pretreatment of filtration leukapheresis donors with colchicine. Blood. in press.

Appendix A

Contract Number: N01-CM-43702

Contractor: Hazelton Laboratories of America, Inc.

Principal Investigator: Charles Bowles

Budget: $408,580

Man Years: 10 (4 = professional, 6 = other)

Objective:

Pre-clinical investigation of leukocyte transfusion, and autologous marrow or peripheral blood stem cell "rescue".

Findings:

The pre-clinical canine model was used to establish that a daily dose of 2×10^8/kg of granulocytes collected by the continuous flow centrifuge is necessary to treat gram-negative sepsis in severely neutropenic dogs (receiving antibiotics and fluids) following injection of 1×10^8/kg Pseudomonas organisms intravenously. A daily dose of 5×10^8/kg of granulocytes is required when WBCs have been collected by adhesion leukapheresis. Studies conducted in the canine bone marrow autograft model (TBI followed by cryopreserved autologous marrow infusion) have established that a dose of 0.5×10^8/kg nucleated marrow cells is needed for hematopoietic reconstitution. Other studies have shown that $2\text{-}3 \times 10^3$ CFU_c/kg of marrow cells and 2×10^3 peripheral blood CFU_c/kg are adequate for reconstitution in this model.

Plan:

The same canine autologous reconstitution model will be used to establish the number of stem cells (indirectly measured by the CFU_c assay) necessary for hematopoietic reconstitution. The canine model will also be used to develop the technical capability to collect adequate numbers of stem cells from the peripheral blood by continuous-flow centrifugation, so as to reconstitute the marrow following TBI. Experiments with dextran sulfate amplification of circulating stem cells will be pursued. We shall continue to examine immune reconstitution using autologous peripheral blood cells versus marrow cells.

Publications:

See main report.

Appendix B

Contract: N01-CM-63810

Contractor: Microbiological Associates

Principal Investigator: Robert Trapani, Ph.D.

Budget: $100,000

Man Years: 3.29 (2.22 = professional)

Objective:

To perform crossmatch studies prior to transfusion of platelets and leukocytes, and to store sera of Pediatric Oncology Branch patients.

Findings:

The POB maintains a serum repository for the serial storage of samples taken from patients undergoing chemotherapy and/or follow-up for treatment of a wide variety of neoplastic disorders. Over 18,000 entries are maintained at -80°C and are catalogued with respect to entry time, patient name and hospital number, disease, and volume of material remaining. Over the past year a number of retrospective studies have been carried out in the following areas, utilizing this important repository.

(a) Chronic graft-versus-host disease (secondary to allogeneic marrow transplantation).

(b) *In vitro* testing of donor-recipient compatibility for WBC and platelet transfusions (leukoagglutinin testing for granulocyte transfusions, and platelet migration inhibition or lymphocytotoxicity assays for platelet transfusions).

(c) Retrospective analysis of patients treated in protected environments to determine evidence of viral infection.

(d) Development of an *in vitro* complement fixation test for serum antigens specific for *Candida albicans* in patients with documented candidiasis in order to develop a diagnostic test for candidiasis (Dr. J. Bennet, NIAID).

(e) Surgery Branch studies on osteogenic sarcoma patients.

(f) Assay of metabolic abnormalities in patients with fever and granulocytopenia (collaboration with Frederick Cancer Research Center).

(g) Assay of core glyoclipid as an indicator of recovery from gram-negative sepsis in neutropenic cancer patients.

Computerization of the serum repository: MBA has undertaken a computerization of the serum repository entries. This capability enables one to order the entries chronologically for each patient and to monitor each entry with an updated account of the remaining volume of each sample at each date. This also permits retrospective analysis of each patient in each disease category since each entry is so listed. A monthly updated report of all remaining entries is issued, enabling close scrutinization of the remaining volume for each patient entry.

Research achieved this year, using this repository, includes:

(a) Completion of a retrospective analysis of over 1000 platelet transfusions to 11 aplastic patients from 48 donors suggesting that HLA matching is a useful but not an infallible predictor of compatible donors for highly alloimmunized recipients.

(b) Development of the platelet migration inhibition assay which, in conjunction with HLA matching, enables one to predict accurately compatible donor-receipient pairs for platelet transfusions. The lymphocytotoxicity and granulocytotoxicity assays may also yield important data in this regard, and the results obtained with these two tests are currently undergoing statistical analysis.

(c) Completion of a study of allograft patients with secondary scleroderma or Sjoegren's-like syndrome.

(d) Completion of a study of chronic graft-versus-host disease in patients with hepatic disorders.

(e) The serum repository is also being utilized to identify the immune defect present in GVHD patients surviving from our earlier allograft program.

(f) A prospective trial of the efficacy of HLA-matched versus unmatched platelet transfusions has commenced, so as further to evaluate prospectively the possible utility of HLA-matched platelet transfusions in cancer patients.

Plans:

The serum repository will be utilized for continuation of the retrospective and prospective trials previously described. In addition, we shall continue to collect samples from patients in anticipation of future clinical investigations. The conclusion of our prospectively randomized trial of HLA-matched vs. mismatched platelet transfusions is critically important to permit adjustment of funding priorities not only intramurally but extramurally as well. The serum repository as well as the *in vitro* tests of compatibility (lymphocytotoxicity, granulocytotoxicity, platelet migration inhibition) represent integral parts of this protocol.

Publications: See main report.

Appendix C

Contract Number: N01-CM-02092

Contractor: UCLA

Principal Investigator: Paul I. Terasaki, Ph.D.

Budget: $378,512

Man Years: 12.9 (8.1 = professional)

Objective:

This is a service contract which provides HLA-typing for patients, families, and blood donors (Clinical Center and Baltimore Cancer Research Center). The contract also provides the computer processing required for the national platelet donor pool.

Findings:

See narrative (06820) concerning numbers of donors HLA-typed and the status of the national donor pool.

Publications:

See main report.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06830-08 PO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Infectious Complications of Malignancy: Prevention, Diagnosis and Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P.A. Pizzo	Senior Investigator	PO C
OTHER:	A.S. Levine	Chief, Ped. Oncol. Branch	PO C
	J.D. MacLowry	Chief, Microbiology Service	CP CC
	F.A. Gill	Assistant Chief, Microbiology Service	CP CC
	F.G. Witebsky	Assistant Chief, Microbiology Service	CP CC
	K. Robichaud	Research Assistant (IPA)	Georgetown Univ.
	E.J. Sussman	Assistant Professor of Nursing	Penn State Univ.
	R.M. Simon	Head, Biostatistical Unit	CO C
	A.E. Jones	Assistant Chief, Dept. Nuc. Med.	NM CC
	J. Bennett	Head, Clinical Mycology Section	LCI, NIAID
	W.J. Hughes	Chief, Infectious Diseases Ped. Dept.	Johns Hopkins U.
	G. Peter	Assistant Professor	Brown Univ.

COOPERATING UNITS (if any)
Georgetown University; CP CC; Penn State University; Biost. Unit; NM CC; LCI (NIAID), Johns Hopkins University; and Brown University.

LAB/BRANCH
Pediatric Oncology Branch

SECTION
Infectious Disease Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	4	2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Infection is the major cause of morbidity and mortality in patients with malignant diseases. We are developing and evaluating the utility of various techniques and procedures to reduce the incidence of severe infection in cancer patients. The primary prophylactic technique under study is the laminar-air-flow type of isolation-filtration unit. We wish to learn whether this unit reduces the incidence of infection during chemotherapy, if patients undergoing such protection can tolerate more intensive chemotherapy, and if they consequently have an improved anti-tumor response. Other interests include the improvement of methods by which occult infectious agents may be detected and eradicated; an evaluation of the acquired germ-free state and the influence of this state on physiologic and metabolic processes; and an evaluation of newer anti-microbial agents. In prospective trials, we are assessing the etiology of febrile episodes in cancer patients, the most appropriate workup for fever in such patients, and the efficacy and toxicity of empiric antibiotic regimens offered for varying periods of time. Other studies concern the RIA and ELISA for candida antigen, core glycolipid in gram-negative sepsis, and P. carinii antigens.

PHS-6040
(Rev. 10-76)

Objectives:

1. To assess the current etiologies of febrile episodes in patients with malignancy, and the relationship of these episodes to cancer treatment, prior infection, degree of host compromise, and granulocytopenia.

2. To determine the most appropriate means for evaluation of fever in the compromised host by assessing invasive and noninvasive diagnostic techniques.

3. To improve methods by which occult infections may be detected and eradicated.

4. To assess the efficacy and toxicity of empiric antibiotic regimens, especially when administered for prophylaxis during periods of profound granulocytopenia.

5. To assess the utility of various techniques and procedures designed to reduce the incidence of infectious complications in patients with neoplastic diseases and in other compromised hosts.

6. To evaluate the utility of the "total protected environment" in reducing the incidence of infection in patients with solid tumors undergoing ablative chemotherapy (with or without autologous bone marrow rescue), and to learn whether such protection permits the administration of more intensive chemotherapy and results in improved tumor responsiveness and prolonged survival.

Methods Employed:

Patients developing a fever are rigorously assessed for a possible infectious etiology and their subsequent course is carefully followed. Patients receiving empiric or prophylactic systemic antibiotics are monitored with pharmacokinetic and metabolic assays for potential toxicity from these antibiotics, and microbiological surveillance for alterations in microbial sensitivity is carried out. Newer diagnostic studies include gallium scintillation scanning, ELISA assays for Candida antigens, assays for Enterobacteriacae anti-core glycolipid and P. carinii antigen, and metabolic screening.

Assessment of the total protected environment [laminar-air-flow-room (LAFR), oral nonabsorbable antibiotic prophylaxis, cutaneous and orificial antisepsis] includes serial patient and environmental microbial surveillance as well as longitudinal assessment of physical compliance and psychological tolerance.

Major Findings:

A. Fever and Antimicrobial Therapy Study

The onset of fever in the granulocytopenic cancer patient may be a manifestation of a serious or life-threatening infection. The appropriate diagnostic evaluation, and the type and duration of specific or empiric antimicrobial therapy, are often unclear. We have prospectively evaluated all Pediatric Oncology Branch patients who develop fever and granulocytopenia since November, 1975. Eligible patients had fever ($\geq$38 C 3 times/24 hrs or > 38.5 C once) and granulocytopenia (PMN's < 500/mm^3). All patients were extensively evaluated and had at least 2 pre-antibiotic blood cultures. Following this evaluation patients were begun on an empiric antibiotic regimen, KGC, consisting of keflin, gentamicin, and carbenicillin. Careful re-evaluation and microbial surveillance were continued.

Three hundred and forty episodes of fever and granulocytopenia were evaluable. Of these, an infectious etiology was clinically and/or microbiologically defined within the first seven days in 179 (53%) of the episodes. The remaining 161 episodes (47%) had no detectable infectious etiology and were, therefore, classified as FUO's. Patients with proven infections and/or FUO were the subjects of further study.

1. FUO episodes: Of the 161 FUO episodes, 86 were associated with granulocytopenia of less than 7 days, while 75 FUO's were associated with granulocytopenia of more than 7 days. In all of the 86 episodes in which granulocytopenia was short (< 7 days), recovery occured without sequelae. The optimal duration of empiric antibiotics for FUO patients with prolonged granulocytopenia has been unclear. In support of continuing empiric antibiotic therapy in such granulocytopenic patients are the potential sequelae which may result from an undiagnosed and inadequately treated infection. On the other hand, the risk of antibiotic-induced organ toxicity, the development of resistant microbial organisms, and the possibility of superinfection argue for shorter treatment schedules when an infectious etiology cannot be diagnosed. A major objective of this study, therefore, is to resolve the "risk versus benefit" dilemma of empiric antibiotic therapy. Of the 75 FUO's with prolonged granulocytopenia 32 were still associated with fever after 7 days of empiric antibiotics had been given; in 43 defervescence had occured. Since the course of these patients might reflect the response (or lack of response) of an occult or undiagnosed infection to the empiric antibiotics, patients with prolonged granulocytopenia (febrile or afebrile) were randomized after 7 days of empiric antibiotics. Patients who had become afebrile but who remained granulocytopenic were randomized either to discontinue or to continue the antibiotics until their granulocyte count rose above 500/mm^3. Patients who were still febrile after 7 days of empiric antibiotics were randomized to either discontinue antibiotics, to continue broad-spectrum antibiotics alone, or to continue empiric antibiotics plus begin empiric amphotericin.

   Of the patients who were afebrile and granulocytopenic, 16 were randomized to continue empiric antibiotics and 17 to discontinue antibiotics. The groups were comparable for all parameters including the duration of granulocytopenia. All of the patients randomized to continue antibiotic therapy until the resolution of granulocytopenia remained afebrile and without infectious sequelae. In contrast, of the 17 afebrile patients randomized to discontinue empiric antibiotics while still granulocytopenic, 7 (41%) became febrile within a median of 2 days of discontinuing antibiotics ($p < .005$). Two of these patients still had no demonstrable source, but again became afebrile upon reinstitution of antibiotics; 1 developed adenitis; 1 cellulitis; 1 pneumonia; and 2 had septicemia (both ultimately fatal). There was no difference in the incidence of noninfectious complications in patients randomized to continue or discontinue empiric antibiotics. Electrolyte disturbances were experienced by both groups and did not increase with prolonged antibiotic therapy. Neither group of patients developed a resistant microbial flora nor superinfections, and the rate of fungal colonization was comparable. It therefore appears that FUO patients with prolonged granulocytopenia who became afebrile during the first 7 days of empiric antibiotics benefit from continued therapy. A similar analysis is underway in the FUO patients who remain febrile after 7 days of empiric antibiotics.

2. Patients with Documented Infection: As noted, in 53% of episodes of fever and granulocytopenia, there was a clinical and/or microbiological source of infection. The sources were most commonly manifested as septicemia, pneumonitis or cellulitis. These patients were studied with the objective of identifying diagnostic tests which might serve to define a possible infectious etiology in FUO patients. These tests have included:

   a) Gallium Scintiscanning - Previous reports have shown that Ga^{67} is taken up in inflammatory sites, and a single case has even suggested diagnostic utility in a patient with aplastic anemia and profound granulocytopenia. Whether this methodology might be useful in neutropenic cancer patients is unknown. We studied 38 patients who presented with fever and granulocytopenia. Of the patients in whom an infectious etiology was defined, fewer than 20% had diagnostic gallium scans. In contrast, more than 70% of the patients with documented infection who were nongranulocytopenic had positive gallium scans. Therefore, gallium scanning is of marginal diagnostic utility in the febrile cancer patient who is also granulocytopenic.

   b) Metabolic correlates of infection: Previous studies have shown predictable alterations in serum iron, zinc, copper, α-1 antitrypsin, transferrin, complement, immunoglobulin, and amino acid profiles in nongranulocytopenic patients who were infected. If similar metabolic patterns are generated in granulocytopenic cancer patients, they might be useful in defining occult infections in patients who are febrile but without an identifiable infectious focus. In collaboration with Dr. Biesel (USAMRI) 50 leukemia patients were studied. Included were non-infected controls, patients who were febrile but not granulocytopenic, patients with proven infections (sepsis or local infections), and granulocytopenic patients with FUO (in whom the ultimate outcome was known). Sera from these patients was assessed in a blind fashion and initial analysis suggests that a combination of changes involving transferrin and the phenylalanine/tyrosine ratio may be predictive of an infectious process.

c) Fungal infections: Morbidity and mortality from fungal organisms in compromised cancer patients is significant and problematic since diagnosis is rarely achieved antemortem. In addition to the randomization of patients with prolonged fever and granulocytopenia to receive empiric amphotericin therapy, antifungal prophylaxis is being employed for granulocytopenic patients with documented Candida colonization of the upper and lower G.I. tract who have been febrile after four days of broad-spectrum antibiotics. The presumed risk of these "colonized" patients is contrasted with patients who have proven deep or superficial fungal infections. In collaboration with Dr. J. Bennett (I LCI), ELISA assays for Candida and Aspergillus antigens are being developed which will be applied to serum samples stored on these defined clinical groupings. Hopefully, this will permit early diagnosis of patients with fungal infections and the opportunity for the early initiation of antifungal therapy.

d) Core glycolipids in gram-negative septicemia: A common antibody to Enterobacteriacae core-glycolipid (CGL) has been identified in animal studies, and the presence of this antibody may be protective during gram-negative septicemia. Should this prove so, passive or active immunization of cancer patients to CGL may improve their outcome if subsequent gram-negative septicemia occurs. As an initial step, we are evaluating patients who survived, or died of, documented gram-negative septicemia for the presence of anti-CGL. Sera from twenty POB patients (along with a control group of gram-positive septicemia patients) are being studied by Dr. Peter (Brown Univ.) for the presence of anti-CGL activity, using hemagglutination, immunofluorescence, and hemolytic assays. An ELISA assay is also being developed. The results will be compared to the patients' clinical course and outcome, and will serve as a basis for possible attempts at immunization.

e) P. carinii antigen: Studies by Hughes et al. have revealed the utility of counterimmunoelectropheresis techniques for the determination of Pneumocystis antigenemia. However, antigenemia was not only described in patients with documented infection, but also a high prevalence was seen among normal children. Refinement of the antigen techniques using RIA and ELISA methodology is now completed. In collaboration with Hughes, we will screen sera from cancer patients who have documented infections or FUO's in order further to clarify the prevalence of P. carinii and the potential application of antigen detection for the early diagnosis of Pneumocystis carinii pneumonia.

f) Treatment of septicemia: The appropriate treatment of patients with documented gram-positive septicemia was studied. Seventy-eight cancer patients with Staph or Streptococcal sepsis over a 10 year period (1968-1977) were analyzed. Patients who remained granulocytopenic (PMN's $< 500/mm^3$) for short periods (< 7 days) recovered without sequelae regardless of the antibiotic therapy employed. However, 7 of 15 patients (47%) who had prolonged granulocytopenia (> 7 days) and who were treated with a single antibiotic developed a second sepsis with a gram-negative bacteria. In contrast, second infections were not observed in granulocytopenic patients treated with a broad-spectrum antibiotic regimen suggesting that such empiric antibiotics may serve a prophylactic role when a cancer patient is granulocytopenic.

g) Staph septicemia: Seventy episodes of S. aureus septicemia which occurred over a nine year period (1968-1976) were reviewed. At the onset of sepsis nearly all patients were febrile, granulocytopenic and had active malignancy. Probable or suspected sites of primary infection were present in 40 episodes (57%). Serious direct complications of staphylococcal sepsis included only three cases of pneumonia and one of myositis. However, second infections by other organisms developed in 16 episodes (24%), resulting in nine nonstaphylococcal infectious deaths during therapy. Endocarditis and osteomyelitis were not observed in any patient and the median duration of anti-staphylococcal therapy was 15 days.

h) Intravenous Bactrim: The parenteral combination of trimethoprim-sulfamethoxazale (Bactrim) has been investigated in approximately 20 patients. We have been able significantly to reduce the fluid load administered with this drug regimen and have found it to be useful and without toxicity in pediatric patients for the treatment of P. carinii pneumonia, as well as for bacteremias resistant to aminoglycoside antibiotics.

Protected Environment Study:

Very intensive chemotherapy may yield responses in metastatic solid tumors or leukemias which are resistant to standard doses of chemotherapy. The major limitation to intensive chemotherapy, however, is severe bone marrow depression and the heightened risk of infection. The use of a Protected Environment (PE) consisting of laminar-air-flow room isolation in conjunction with nonabsorbable oral antibiotics, cutaneous antisepsis, and a diet with a low microbial burden has been shown to reduce the incidence of infection related to prolonged granulocytopenia. The present study addresses the following questions: First, can patients, including young children, tolerate prolonged periods of isolation and comply with the PE regimen? Secondly, would the PE regimen successfully reduce the incidence of infection in young patients with solid tumors as it has in adults with leukemia and permit the administration of more intensive chemotherapy? And finally, will such intensive drug regimens improve the remission rate and/or duration of response? Patients with diverse tumors refractory to standard doses of chemotherapy have been studied initially. Patients received varying 3-4 drug combinations which included vincristine, methotrexate, adriamycin, actinomycin, ara-C, 6TG, M-CCNU, DTIC, and/or L-PAM. In addition, each regimen included high-dose cyclophosphamide in dose ranging from 135-200 mg/kg given over 3-4 days. These regimens resulted in periods of absolute granulocytopenia (< 500 PMN's/mm^3) ranging from 22-66 days. Patients whose bone marrow had been cryopreserved prior to relapse were eligible to receive marrow re-infusion following this intensive drug regimen. Autologously transplanted patients were randomized to receive the intensive drug therapy in either a PE or regular hospital setting. If cryopreserved bone marrow was not available, patients were nonrandomly allocated to a PE. Thirty-nine patients (8 osteosarcoma, 12 lymphoma, 4 rhabdomyosarcoma, 8 Ewing's, 5 neuroblastoma, 2 leukemia) were treated in a LAFR and 25 patients (4 osteosarcoma, 7 lymphoma, 4 rhabdomyosarcoma, 5 Ewing's, 3 neuroblastoma, and 2 leukemia) received similar chemotherapy in a conventional ward setting. Because of the study design, fewer PE patients received autologous bone marrow infusions and, therefore, had a longer duration of post-therapy granulocytopenia compared to the control patients. The following general conclusions can be drawn: First, all patients completed the therapeutic regimen and no patient had to be removed from a PE (patients as young as 2 years were able to tolerate and comply with the prophylactic decontamination program for periods of approximately 6 weeks). Secondly, nearly all patients became febrile after the onset of granulocytopenia. The duration of specific and empiric systemic antibiotic therapy was comparable in PE and control groups. A clinical and/or microbiological source for the fever was found in 1/3 of the PE and 1/2 of the control patients. The use of autologous marrow rescue did not alter this pattern of infectious complications. However, when the incidence of infection was corrected for the duration of granulocytopenia, a significant difference ($P < .05$) was observed between PE and control patients. In addition to infectious complications, noninfectious sequelae were significant (especially cardiac myonecrosis) and resulted in a mortality of 14%. Hence, even if the PE regimen is successful in reducing the morbitity and mortality due to infection, extramedullary toxicity presents an equally formidable limitation to intensive chemotherapy. Regarding tumor response, there was no overall difference in the response rate between PE and control patients. Of interest is that approximately half of these refractory patients had either a complete or partial response to the intensive drug regimens, which is encouraging since refractory tumors of these types almost invariably progress on standard dosage schedules. However, the median duration of this response was brief, being only 2 months. Nonetheless, approximately 10% of all the patients enrolled in this study have had prolonged remissions following intensive chemotherapy, although the durable remissions have been seen only in patients with non-Hodgkin's lymphoma and rhabdomyosarcoma. The lack of utility of the PE for the majority of these refractory patients reflects our relatively ineffectual chemotherapy for this group of patients rather than a lack of value for this sophisticated isolation technology. The use of high-dose regimens may, however, prove more useful early in the treatment schedule and prior to the development of drug resistance in poor prognosis patients.

Significance to Biomedical Research and the Program of the Institute:

One of the major sequelae of chemotherapy is bone marrow depression resulting in significant granulocytopenia. Such granulocytopenia significantly increases the incidence of life-threatening infectious complications in the cancer patient. Consequently, supportive care of the patient is essential if the potential benefits of chemotherapy are to be achieved. This implies not only an understanding of the natural history of infectious complications, but also the development of treatment schedules which provide maximum benefit and safety for the patient. Since our studies suggest that escalation of chemotherapy may improve the response rate for patients resistant to conventional doses of chemotherapy, and since the risk of infections is a major impediment to such intensification, the development of programs which reduce the incidence of infection during periods of granulocytopenia is essential. Our studies to date suggest that regimens using prophylactic antimicrobial therapy may be helpful in lessening the infectious morbidity and mortality related to protracted granulocytopenia. This affords the opportunity to explore new chemotherapeutic strategies which may provide a higher therapeutic index.

Proposed Course:

Our studies of the role of prophylactic empiric antibiotics and antifungal therapy in patients with granulocytopenia will be continued. Over the next year we should be able to describe appropriate treatment strategies for high-risk patients with fever and prolonged granulocytopenia. By employing a variety of assays for microbial antigens, we should be able to identify patients who are at high-risk for infection but who cannot be diagnosed by conventional means. This should permit earlier intervention and more effective therapy. As an extension of our initial observations on the utility of prophylactic systemic antibiotics prior to the onset of fever, such prophylaxis will be explored in granulocytopenic patients in a randomized prospective trial. Studies of the laminar air-flow protected environment will also be continued, with emphasis on intensive treatment of poor-risk tumors in such rooms before pharmacologic resistance occurs.

Publications:

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2. Ihde, D.C., Roberts, W.C., Mahr, K.C., Brennan, M.D., McGuire, W.P., Levine, A.S., and Young, R.C.: Cardiac candidiasis in cancer patients. Cancer, in press.

3. Kramer, B.S., Hernandez, A.D., Reddick, R.L., and Levine, A.S.: Cutaneous infarction: Manifestation of disseminated mucormycosis. Arch. Derm. 113: 1075-1076, 1977.

4. Ladisch, S. and Pizzo, P.A.: *S. aureus* sepsis in children with cancer. *Pediatrics* 61: 231-234, 1978.

5. Levine, A.S. and Pizzo, P.A.: Management of infections in children with cancer. *Pediatric Annals*, in press.

6. Pizzo, P.A.: Fever of unknown origin. In Hoekelman, R.A., Blatman, S., Brunell, P.A., Friedman, S.B., and Seider, H.M. (Eds.): *Principles of Pediatrics: Health Care of the Young*. McGraw Hill, 1978.

7. Pizzo, P.A., Ladisch, S., Simon, R.M., Gill, F., and Levine, A.S.: Increasing incidence of gram-positive sepsis in cancer patients. *Med. and Ped. Oncol.*, in press.

8. Pizzo, P.A., Ladisch, S., and Witebsky, F.G.: Alpha-hemolytic streptocci: Clinical significance in the cancer patients. *Medical & Ped. Oncol.*, in press.

9. Pizzo, P.A., and Levine, A.S.: The utility of protected environment regimens for the compromised host: A critical assessment. In Brown, E.B. (Ed.): *Progress in Hematology* Vol. X, Grune and Stratton, 1977, 311-332.

Appendix A

Contract Number: N01-CM-53832

Contractor: The University of Texas System Cancer Center: M. D. Anderson Hospital

Principal Investigator: Gerald P. Bodey, M.D.

Budget: $650,000

Man Years: 11 (Professional - 2; Other - 9)

Objective:

To assess the utility of protected environments in permitting the administration of high-dose chemotherapy to adult patients with non-Hodgkin's lymphoma, differentiated sarcomas, and various carcinomas.

Findings:

Results of the contractor's clinical studies in non-Hodgkin's lymphoma, sarcoma, and carcinoma of the breast have been encouraging in that they confirm that the protected environment (laminar-air-flow-room) reduces the incidence of infection in patients receiving high-dose chemotherapy. While there is a suggestion both in the lymphoma and sarcoma studies that higher doses of therapy produce a higher complete remission rate and/or a more durable responses, the data are not sufficiently firm due to insufficient stratification of patient-risk factors. A number of changes in protocol design have been suggested as a result of review of this contract, and the revised protocols consequent to these suggestions should permit the rapid accummulation of definitive data.

Plan:

The sarcoma study will be discontinued since it is not possible to accrue a statistically adequate patient-sample size in any one of the differentiated sarcomas; rather than accepting a heterogeneous sarcoma population, this study will be replaced by a high-dose approach to small cell carcinoma of the lung. The studies will thus consist of a randomized trial of chemotherapy in metastatic carcinoma of the breast; a non-randomized, historically controlled study of extensive oat cell carcinoma of the lung; and a historically controlled study of the diffuse histocytic lymphoma. With respect to the breast carcinoma study, the control group is comprised of patients receiving conventional dose chemotherapy as out-patients. In all three studies, patients are to be carefully stratified with respect to known prognostic factors. It is hoped that within two-three years, this contractor will be able to provide definitive information on the utility of protected environments in permitting higher doses of chemotherapy in these poor-risk patients than would otherwise be the case, as well as demonstrating whether these higher dose schedules are associated with more durable antitumor responses.

Publications:

1. Bodey, G. P., and Rodriguez, V.: Protected environment-prophylactic antibiotic programs: Microbiologic studies. In: Bodey, G. P. (Ed.) *Clinics in Hematology*. W. B. Saunders Publishers, London, Vol. 5, pp. 395-408, 1976.

2. Feld, R., and Bodey, G. P.: Infections in patients with malignant lymphoma treated with combination chemotherapy. *Cancer* 39: 1018 1025, 1977.

3. Rodriguez, V., Cabanillas, F., Burgess, M.A., McKelvey, E.M., Valdivieso, M and Bodey, G. P.: Combination chemotherapy with "CHOP-BLEO" in advanced non-Hodgkin's malignant lymphoma. *Blood* 49: 325-333, 1977.

4. Keating, M., Rodriguez, V., and Bodey, G.: Protected environments and microbial suppression in cancer therapy. *Amer. J. of Clin. Nutr.* 30: 1904-1910, 1977.

5. Bodey, G. P., Rodriguez, V., Chang, H. Y., and Narboni, G.: Fever and infection in leukemic patients. A study of 494 consecutive patients. *Cancer* 41: 1610-1622, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06840-03-PO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Tumor Immunology and Immune Surveillance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.G. Poplack, Senior Investigator	PO C
Other:	S. Ladisch, Clinical Associate	PO C
	G. Reaman, Clinical Associate	PO C
	R.M. Blaese, Head, Cellular Immunology Section	MET C
	S. Broder, Senior Investigator	MET C
	A. Muchmore, Senior Investigator	MET C
	D. Nelson, Senior Investigator	MET C
	E.C. Lawrence, Clinical Associate	MET C
	W. Pichler, Visiting Fellow	MET C
	D. Mann, Senior Investigator	MET C
	J. Smythe, Department of Medicine	Royal Marsden Hosp.
	J. Donlon, Clinical Associate, Hematology Service	CP CC

COOPERATING UNITS (if any)
Metabolism Branch (NCI); Department of Medicine, Royal Marsden Hospital; and Clinical Pathology Department (CC)

LAB/BRANCH
Pediatric Oncology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 5	PROFESSIONAL: 3	OTHER: 2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this project, mechanisms of normal cellular immunity and tumor immunology are studied: immunological surveillance of cancer patients and the effects of chemotherapy and immunotherapy on the immune response are explored. We are characterizing acute lymphocytic leukemia according to the immunological subtypes of lymphoblasts and the capacity of lymphoblasts to express immunoregulatory helper and/or suppressor functions in vitro. Possible correlation between lymphoblast cell surface markers with various biochemical characteristics is being pursued. The role of B cell alloantigens in lymphoblastic leukemia is also under investigation. Particular emphasis is placed on immunologic study of the monocyte-macrophage system (including development of new clinical assays of human monocyte function) in malignancies and immunodeficiency diseases, in the newborn, and in the histiocytoses. We are also investigating serum suppressors of cellular immunity in cancer patients, and the role of immune adjuvants and their ability to stimulate granulocyte recovery following marrow-suppressive chemotherapy.

Project Description:

Objectives:

1) To assess the effect of chemotherapy and immunotherapy on the host immune response.

2) To develop methods of distinguishing between immunological subtypes of leukemic lymphoblasts and further to characterize such subtypes.

3) To define possible correlations between immunological cell surface markers and various biochemical characteristics of leukemic lymphoblasts.

4) To assess whether acute leukemic lymphoblasts are capable of manifesting immunologic "helper" and/or "suppressor" function, using *in vitro* immunologic assays.

5) To explore the role of the monocyte-macrophage in human disease states and to assess the functional heterogeneity of this cell system during normal maturation.

6) To determine the relationship of B cell alloantigens to leukemic lymphoblasts.

7) To determine the effect of chemotherapy on the recovery of granulocytes following chemotherapy-induced granulocytopenia and to investigate the mechanism(s) of granulocyte recovery.

8) To study the mechanism(s) of lipid-induced plasma suppression of immune function.

Methods and Major Findings:

A. Immune surveillance of leukemia patients

Serial immune evaluation has been performed on patients who, while on Protocol 72-1 (initial treatment of acute lymphoblastic leukemia), received maintenance chemotherapy according to one of three treatment arms designed to compare the effectiveness of chemotherapy versus chemotherapy plus immunotherapy. Those patients who received BCG immunotherapy developed a vigorous skin test response to PPD; however, all other recall skin test responses remained stable throughout the study. *In vitro* lymphocyte responses to specific antigens and nonspecific mitogens progressively declined with time. These findings suggest that the recognition and memory functions of the immune system remain intact during chemotherapy, but the proliferative response is impaired. We have extended this serial immune evaluation in patients who successfully completed chemotherapy on the 72-1 protocol. Examination of afferent and efferent cellular immune function revealed that within one month following the cessation of chemotherapy, immune responsiveness of patients returns to normal. Six to twelve months later, immune responsiveness is also intact. Thus, the immunosuppression which occurs during chemotherapy is rapidly reversed following cessation of treatment.

B. Immune evaluation of Ewing's sarcoma patients undergoing total body irradiation

A study is underway to determine the effect of total body irradiation (TBI), as employed in the treatment of Ewing's sarcoma, upon the immune system. Particular attention is given to the effects of TBI upon monocyte-macrophage function. The macrophage is classically described as a radio-resistant cell. Efforts are being made to determine whether TBI influences either circulating monocyte or tissue macrophage function.

C. Immunologic characterization of T cell sub-sets in human T cell acute lymphoblastic leukemia

Recently it has been found possible to distinguish between different T cell sub-sets on the basis of whether these cells rosette with either IgM or IgG-coated ox red blood cells. This identification of T cell sub-sets, i.e., T cells which bear receptors for either IgM (Tμ) or IgG (Tγ), has been found to be of functional significance, since Tμ cells have suppressor functions and Tγ cells can mediate cytotoxicity. We have characterized the T lymphoblasts of ALL patients and have found cells with either Tμ or Tγ characteristics. More importantly a number of patients have been identified who possess both Tμ and Tγ receptors, suggesting that these leukemic cells represent a more undifferentiated lymphoblast. Studies correlating these findings with lymphoblast biochemical markers and various *in vitro* immunological functions (i.e. suppression, cytotoxicity) are underway.

D. Correlation of 5' nucleotidase activity with certain cell surface markers in acute lymphoblastic leukemia

The ectoenzyme, 5' nucleotidase (5'N), has been demonstrated on the surface of human lymphocytes. We have studied the activity of 5'N on the lymphoblasts of patients with acute lymphoblastic leukemia and have correlated these findings with immunological cell surface markers. The peripheral lymphoblasts of 15 previously untreated patients with ALL and the lymphocytes of normal donors were assayed for 5'N. 5'N activity was assessed by quantitating the dephosphorylation of the radioactive substrate, adenosine 5'MP[^{14}C(U)], in a reaction mixture containing 4x10^{6} cells, following a 30 minute incubation and LaCl$_3$ precipitation. Erythrocyte (E) and erythrocyte antibody complement (EAC) rosettes and surface immunoglobulin were evaluated by standard techniques. Cytocentrifuge preparations of cell suspensions were examined to determine the morphology of rosette-forming cells and the percentage of lymphoblasts forming E or EAC rosettes.

The lymphoblasts of 8 patients formed E rosettes (T lymphoblasts) and the remaining 7 patients had null lymphoblasts. The mean 5'N activity of T lymphoblasts (0.004 nmoles AMP/10^6 cells/min.) was significantly less than that of either normal lymphocytes (0.043 nmoles AMP/10^6 cells/min.) ($p < .001$) or null lymphoblasts (0.025 nmoles AMP/10^6 cells/min.) ($p = .001$).

These findings offer additional biochemical evidence for heterogeneity in acute lymphoblastic leukemia and provide a method for distinguishing between immunological subtypes of this disease.

E. Adenosine deaminase in lymphoblasts

We have shown that adenosine deaminase (ADA) levels in blast cells of patients with acute lymphocytic leukemia are elevated when compared to levels in normal lymphocytes. This observation prompted us to study the possibility of a correlation between ADA activity and lymphoblasts differentiated on the basis of their cell surface markers. Lymphoblasts from untreated patients with ALL were evaluated for the presence of sheep erythrocyte and compliment receptors using standard E and EAC rosetting techniques. In addition, sonicated extracts of these Ficoll hypaque-separated lymphoblasts were assayed spectrophotometrically for ADA activity. Of 23 patients tested, 9 had T lymphoblasts (E rosette positive) and 14 had null lymphoblasts (E and EAC rosette negative). The mean ADA activity was 3 times higher in T lymphoblasts than in null lymphoblasts. Thus, there is a significant ($p < .02$) positive correlation between elevated ADA levels and T cell-ALL. This method of distinguishing between immunological subtypes of lymphoblasts is potentially of prognostic and therapeutic value.

F. Terminal transferase in ALL

We are performing studies of terminal transferase in lymphoblasts from patients with acute lymphoblastic leukemia. These studies involve the use of a standard enzymatic technique as well as an immunofluorescent technique developed by Dr. Bollum (National Naval Medical Center). Results to date have demonstrated that a significant percentage of ALL patients have lymphoblasts which are negative for terminal transferase. A preliminary correlation of terminal transferase activity in leukemic lymphoblasts with patient prognosis suggests that those individuals who are TdT negative may have an exceptionally poor prognosis. Studies to correlate TdT activity with ADA, 5'nucleotidase and T cell sub-set information (see sections C, D, E above) are currently in progress.

G. Suppressor activity in T-cell leukemia

Since it has been shown that T lymphocytes are capable of manifesting either "helper" or "suppressor" modulatory effects on in vitro immunoglobulin byiosythesis, we are studying whether lymphoblasts from patients with T cell-ALL are capable of this type of immunoregulatory activity. Studies of one patient with T cell leukemia have indeed demonstrated the presence observed. The mechanism of this phenomenon is as yet unclear, and is the subject of current investigation.

J. Stimulatory effect of BCG on granulocyte recovery

We have conducted studies designed to determine whether immunotherapy stimulates the recovery of granulocytes following leukopenia-inducing chemotherapy. Using an animal (mouse) model, we found that pretreatment with the immunoadjuvant BCG resulted in a more rapid recovery of the leukocyte count following treatment with cyclophosphamide. We have also found that the more rapid recovery in BCG-pretreated mice occurs on the basis of stimulation of the granulocyte series alone.

Recently we have studied the mechanism of this BCG-induced acceleration of myeloid recovery after cyclophosphamide-induced marrow suppression. Seven week old male C57BL/6 mice received intraperitoneal BCG or saline 8 days prior to a single dose of cyclophosphamide. Serum levels of colony stimulating factor (CSF) and the number of marrow granulocyte colony-forming units (CFU-c) were assessed during recovery from leukopenia. Both CSF levels and marrow CFU-c numbers were markedly increased in the BCG-treated mice during the recovery period, suggesting that rapid recovery from cyclophosphamide-induced marrow suppression may be due to BCG-induced stimulation of CSF and an increase of marrow CFU-c.

K. Immunological investigation of the histiocytic syndromes

Since the monocyte-macrophage is the principle cell involved in the histiocytic syndromes, we have investigated patients with these disorders in an attempt to learn whether the monocyte-macrophage system functions abnormally. Four patients with familial erythrophagocytic lymphohistiocytosis (FEL) (a fatal familial syndrome characterized by abnormal accumulation of non-malignant histiocytes) were found to be anergic to recall skin test antigens. *In vitro* evidence of abnormal cellular immune function included depressed lymphocyte proliferative responses to specific antigens, and impaired monocyte-mediated cytotoxic function. We have found that plasma from patients with lymphohistiocytosis suppresses the responses of normal lymphocytes. On further investigation, it was found that the suppressive plasma was hyperlipidemic; moreover, the degree of *in vitro* immunosuppression by these plasmas correlated with the degree of lipid elevation. Since an association between hyperlipidemia and suppression of *in vitro* immune responsiveness has recently been reported, hyperlipidemia may be in part responsible for the immune defects in these patients.

We have also evaluated 5 children with the more common childhood reticuloendotheliosis, histiocytosis X. Two of the 3 patients tested were anergic. Four of 5 had virtually absent *in vitro* lymphocyte blastogenic responses to specific antigens when tested in normal plasma, while responses to allogeneic cells and non-specific mitogens were normal or only slightly depressed. In addition, all 5 patients were hyperlipidemic (plasma triglycerides: 160-1000 mg%, normal < 150 mg%); incubation of of suppressor activity. Interestingly, this suppression of immunoglobulin biosynthesis required the cooperation of a normal T cell. This is the first documentation in man of suppression of this type (requiring the interaction of 2 different cooperating T cells) although such interaction had previously been demonstrated in animal systems. The possibility that these in vitro findings have a clinical correlate is suggested by recent observations of immunoglobulin abnormalities in patients with acute lymphoblastic leukemia.

H. B Cell alloantigens

We have been studying the distribution of B cell alloantigens on the lymphoblasts of patients with ALL. A comparison of the antigen typing of lymphoblasts of patients and their siblings has demonstrated the presence of different B cell alloantigens on leukemic lymphoblasts than are present on the normal lymphocytes of either the patient or the siblings. Further studies which may allow us to determine the genetics of these B cell alloantigens are in progress.

I. Monocyte-mediated antibody-dependent cellular cytotoxicity (MMADCC)

We have developed an assay of antibody-dependent cellular cytotoxicity which depends exclusively on the monocyte as the effector cell and therefore provides a convenient test of monocyte function. This assay has detected monocyte dysfunction in patients with the Wiskott-Aldrich syndrome and children with familial erythrophagocytic lymphohistiocytosis. In addition, patients with aplastic anemia who are monocytopenic have defective MMADCC. Exploration of the mechanism of monocyte mediation of ADCC is in progress. We have been particularly interested in understanding the role of anti-target cell antibody in determining which effector cells are capable of mediating ADCC. We evaluated human erythrocyte lysis by both monocyte-containing human peripheral blood mononuclear cells (PBMC) and monocyte-depleted peripheral blood lymphocytes (PBL). Lysis of TNP-modified human type B erythrocytes was evaluated in the presence of human anti-B antiserum, human anti-CD serum, and monkey, rabbit and goat anti-TNP antisera. Human PLB mediated the lysis of TNP-modified type B, Rh(D) positive erythrocytes in the presence of human anti-CD and all three anti-TNP antisera. However, PBL did not mediate lysis when anti-B serum was utilized. These experiments confirmed the monocyte specificity of MMADCC using anti-B serum and B positive erythrocytes. In addition, the observation of ADCC by PBL for human erythrocyte targets in the presence of xenogeneic and allogeneic anti-target sera demonstrated that anti-target cell serum is critically important in determining which mononuclear effector cell mediates ADCC.

We have also studied the effect of steroids upon MMADCC and upon the spontaneous cytotoxicity of chicken erythrocytes, a non-antibody dependent monocyte-mediated cytotoxicity system. Preliminary studies have shown a differential effect of in vitro incubation with glucocorticoid upon these processes: Whereas hydrocortisone does not inhibit MMADCC, profound steroid-induced inhibition of non-antibody mediated monocyte lysis was normal lymphocytes in these hyperlipidemic plasmas resulted in suppression of blastogenic responses, particularly to specific antigens and allogeneic cells.

The finding of defects in cellular immunity and the presence of a plasma suppressor factor associated with hyperlipidemia are similar to results in patients with FEL. In contrast to FEL, however, the patients with histiocytosis X had normal monocyte-mediated cytotoxic effector function, and the abnormalities in immunoglobulin levels and specific antibody synthesis seen in FEL were absent.

These observations indicate the presence of an apparently isolated defect in cellular immunity in histiocytosis X, in contrast to defects in both humoral and cellular immunity and evidence of monocyte/macrophage dysfunction in FEL. The possible role of hyperlipidemia in the immunological defects and the pathogenesis of these two childhood reticuloendothelioses remains to be elucidated.

L. Lipid induced immunosuppression

We have investigated the association of serum lipids and immune function and have confirmed an association between hyperlipidemia and suppression of *in vitro* immune responsiveness. The observations in section K led us to examine the effect of intralipid (IL), a fat emulsion widely utilized in intravenous hyperalimentation regimens, upon *in vitro* lymphocyte function.

The blastogenic response of normal human peripheral blood leukocytes (PBL) in the presence of 100-1000 mg% IL was determined. At these IL concentrations, although responses to non-specific mitogens were virtually unaffected, responses to specific antigens and allogeneic cells were markedly inhibited in a dose-related manner; 1000 mg% IL caused up to 90% inhibition. Short-term preincubation with IL ($\leq$ 24 hrs.) caused inhibition reversible by cell washing. However, long-term preincubation (3-5 days) or higher IL concentrations (2000 mg%) resulted in increasingly irreversible inhibition. To extend these observations, plasma was obtained from patients receiving IL infusions as part of a hyperalimentation protocol. Post-infusion plasma caused up to 100% suppression of the response of normal PBL to specific antigens and allogeneic cells, with pre-infusion plasma used as a control. The inhibitory effect of IL was shown not to be due to lymphocytotoxicity, alterations in the kinetics of the blastogenic response, or changes in the optimal concentrations of stimulant. Thus, at concentrations achieved *in vivo* after intravenous infusion, IL depresses *in vitro* lymphocyte blastogenesis. These data raise the possibility of an adverse effect of IL on immunological function in patients receiving IL hyperalimentation.

Significance to Biomedical Research and the Program of the Institute

Our immunological studies have helped to assess the effects of chemotherapy and immunotherapy on the immune system of patients with malignancy. Since in recent years, it has been shown that the macrophage-monocyte system plays a central role in the host defense against tumor and infection, the development of assays of human monocyte function and the confirmation of their ability to detect clinical abnormalities are of singular importance in permitting further understanding of the monocyte-macrophage system in human disease. Our studies on characterization of leukemic cells using immunological and biochemical markers may be of prognostic and therapeutic importance. Evaluation of the immunologic function of these cells, e.g. immune suppression, may lead to new approaches in the chemotherapy and immunotherapy of patients with acute lymphoblastic leukemia. Studies on the ability of immunotherapeutic agents potentially to stimulate the recovery of granulocytes following chemotherapy-induced marrow suppression may be of value in the treatment of patients with malignancies. Our observations on lipid-induced immune suppression (histiocytosis, in vitro effect of intralipid) reveal a physiologic interaction not hitherto appreciated, and bear implications for clinical hyperalimentation.

Proposed Course

The effort to characterise biochemically and immunologically the lymphoblasts from patients with acute lymphoblastic leukemia will be expanded. An attempt will be made to correlate the various findings regarding cell surface markers and immunological sub-sets with the biochemical markers discussed above. The prognostic and therapeutic relevance of these findings will also be pursued. Investigation into the role of the macrophage-monocyte in both normal and disease states will also include studies designed to examine the functional heterogeneity of this system expressed during maturation. Our findings regarding granulopoietic stimulation by BCG will be amplified by the study of a variety of immune adjuvants.

We plan to evaluate in animal systems the potential therapeutic (i.e., hematopoietic reconstitution) efficacy of in vitro incubation of leukemic bone marrow with specific anti-leukemic antisera and the subsequent infusion of such treated marrow into the chemotherapy-ablated host. Finally, we will continue immune surveillance of patients with malignancies, with special emphasis on assessment of monocyte-macrophage function in these individuals.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06850-08 PO

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October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

DNA and RNA Tumor Viruses: Molecular Mechanisms of Infection and Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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16	10	6

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the genetic organization and expression of SV40 and adenovirus 2 by an analysis of the SV40 and Ad2 genomes contained within Ad2-SV40 hybrid virus DNA molecules. The hybrid viruses are also employed as models for understanding the behavior of integrated genomes. We are also analyzing the behavior of SV40 and Ad2-transformed or lytically infected cells as a function of the molecular conformation of viral nucleic acids within these cells, and as a function of events which may control (or be controlled by) viral replication, transcription, and translation. Secondly, we are analyzing the state and expression of the endogenous AKR murine leukemia virus genome in mice with varying expression of leukemia and virus production. A further objective is to explore the evolution, transmission, and relation to leukemogenesis of various murine RNA viruses. Finally, this laboratory is concerned with production and purification of Epstein-Barr virus DNA and the use of this reagent to explore molecular mechanisms of infection and oncogenicity associated with this human herpes virus. Studies of cell lines from human lymphomas include surface markers, karyotypes, presence of and infectibility by EBV. growth characteristics, and morphology. New EBV strains from African Burkitt's (true tumor strains?) are also studied.

Project Description/

Objectives:

1) To study the genetic organization and expression of Simian Virus 40 (SV40) and human Adenovirus 2 (Ad2) by an analysis of the varying segments of the SV40 and Ad2 genomes contained within Ad2-SV40 hybrid virus DNA molecules. Such studies employ biologic, biochemical, biophysical, and electron microscopic techniques to achieve genetic maps of these animal tumor viruses. The hybrid viruses are also employed as models for understanding the behavior of integrated genomes, the phenomenon of integration appearing central to the molecular mechanism of viral oncogenesis.

2) To analyze the behavior of SV40 and Ad2-transformed or lytically infected cells as a function of the molecular conformation of viral nucleic acids within these cells, and as a function of events which may control (or be controlled by) viral replication, transcription, translation and maturation. The early antigens of SV40 and their role in cell transformation and tumor rejection are of particular interest.

3) To improve the production and purification of Epstein-Barr virus (EBV) DNA and to use the reagent to explore the molecular mechanisms of infection and oncogenicity associated with this human herpes virus; to identify more precisely the probable cell of origin of Burkitt's lymphoma; to attempt to provide better diagnostic criteria of lymphomas by providing data at a functional level which relates to specific lymphoma sub-types; to compare EBV genome-positive cell lines with EBV genome-negative lines and to determine whether differences relate simply to the presence of the genome, or reflect a difference between cells which can be infected by EBV *in vivo* and cells which cannot; to determine the relevance of different strains of EBV to the range of EBV-associated diseases and to associate biological differences in virus strains with biochemical differences.

4) To enhance our understanding of the natural history of murine leukemia by biochemically analyzing various murine leukemia viruses, both those occurring naturally as well as laboratory-derived strains. We believe that such analyses will not only improve our understanding regarding leukemia-causing viruses, but also our understanding regarding the nature of high-, low-, and non-leukemic strains of mice. A further objective is to explore the evolution and transmission of these viruses, using cells from a variety of wild rodents and related species.

5) To understand the mechanism of action of halogenated pyrimidines, e.g., IUdR (a drug which activates production of various viruses, including C-type RNA virus, from non-producer cells) and thioacetamide (a carcinogen which very rapidly and efficiently produces hepatic carcinoma in rats).

6) To identify transformation-specific proteins induced by RNA tumor viruses and to study their properties, with the hope of obtaining further insight into the mechanism of carcinogenesis by retroviruses.

Methods Employed:

Procedures for the isolation of highly purified viral and cell macromolecules are continuously improved and new techniques for hybridization of viral and cellular nucleic acids are developed. A variety of techniques which permit quantitative comparison of nucleotide sequences are in use. This laboratory routinely employs equilibrium density centrifugation, analytical ultracentrifugation, electron microscopic heteroduplex mapping, enzymatic fragmentation and strand separation of viral DNA, gel electrophoresis, hydroxyapatite chromotography, and the *in vitro* synthesis of viral DNA probes. Nucleotide sequencing of specific viral RNA transcripts is available. *In vitro* protein synthesis using cell-free systems is undertaken. An extensive virus purification and tissue culture facility is maintained; plaque assays and CF or IF tests for viral-specific antigens are performed. Radiolabelled proteins are analyzed via autoradiography or fluorography; fingerprint analysis of the tryptic peptides of purified proteins is performed by a two-dimensional procedure involving high-voltage electrophoresis and thin-layer chromatography. Mouse breeding experiments are performed in association with studies on the genetics of RNA tumor viruses. Immunological methods include study of surface immunoglobulins and rosette-forming cells utilizing a variety of reagents including those made from sheep erythrocytes, antibody and complement, and also from fluoresceinated salmonella typhi. Reagents made from purified complement components represent an essential part of the methodology. Immunofluorescent assay is used to detect the presence of EBV virus in tumor and tumor-derived cell lines, and flow microfluorimetry for characterization of distribution of cell size, DNA content, and protein content within the cell population.

Major Findings:

A. Studies of Naturally Occuring Murine Leukemia Viruses

Utilizing a [^{3}H] viral DNA probe synthesized from naturally occuring AKR murine leukemia virus RNA, we have established that a good correlation exists between the completeness of the viral sequences in the host genome, the number of copies of the viral-specific sequences, leukemogenesis, and the production of virus by various mouse strains. Furthermore, we have demonstrated that a major virus-inducing locus, *Akv-1*, is a viral structural gene. We have now extended this study by determining the number of copies of the AKR virus genome integrated into the cellular DNA after exogenous infection of NIH mouse, AKR mouse, and rat cells in tissue culture. NIH mouse cells, which lack a portion of the viral genome (referred to as Gross-AKR specific sequences), incorporated three to four copies of these sequences per haploid genome. AKR cells, in which the Gross-AKR specific sequences are already present as three to four copies per haploid genome, did not show any distinct change in copy number after infection. Rat cells, which lack DNA sequences homologous to murine leukemia virus, incorporated one copy of the viral genome per haploid genome. It is inferred that the presence of viral sequences may affect the efficiency of integration of exogenous provirus, and that there may be a limit to the number of copies that can be inserted.

b) From a non-producer mouse cell line (AKR-2B) developed from AKR mouse embryos, ecotropic virus can be readily activated after treatment with 5-iododeoxyuridine. However, amphotropic viruses cannot be so activated, which indicates that there are different regulatory mechanism(s) for the induction of these viruses.

B. Electron Microscopic Study of Naturally Occurring Murine Leukemia Viruses

We have studied 50-70S RNAs of naturally occurring ecotropic, xenotropic, and amphotropic murine leukemia viruses by electron microscopy. All of these viruses have 5' to 5' dimer structures. Like other xenotropic viral RNAs obtained from cats and baboons (RD 114 and BKD, respectively), xenotropic virus from the NZB mouse has an unusually high melting temperature (the temperature at which dimers become monomers).

C. Development of Cell Lines from Type-C MuLV Sequence-Negative Mouse Strains

We have developed several cell lines from various *Mus* species which are of great importance in that they support the growth of ecotropic, xenotropic and amphotropic viruses equally well.

D. Mechanism of IUdR Activation of Virus

Halogenated pyrimidines (e.g., IUdR) activate C-type virus production from normal cells of a wide variety of animals in tissue culture. The pyrimidines become incorporated into the DNA of these cells, but the mechanism by which they activate virus is not known. In a normal (non-producer) cell line derived from AKR embryos, about 30-40% of the viral sequences are present in cellular RNA. However, after treatment with IUdR, 100% of the ecotropic viral-specific sequences are expressed in cell RNA. To understand the mechanism of the IUdR effect, we have attempted to determine whether viral-specific sequences are newly and exclusively transcribed after IUdR treatment, or whether this drug increases the overall transcription of cellular DNA with integrated viral sequences comprising one part. Our hybridization experiments with $[^3H]$ unique mouse cellular DNA and RNA indicate that IUdR increases the overall transcription of cellular DNA in the AKR mouse, i.e., sequences that had not been transcribed now are. We could not, however, detect any alteration of the transcription pattern of the sequences that were being transcribed before IUdR treatment, except that their rate of synthesis and/or accumulation in the cell increased.

E. Mechanism of Action of Thioacetamide

We have found that treatment of rats with thiacetamide (TAA), a liver carcinogen, results in a greatly increased production of total mRNA and specifically albumin mRNA (mRNAalb). The amount of mRNAalb was indirectly quantitated by immunoprecipitation of enzymatically-synthesized albumin using antialbumin serum. It was found that about 10% of the mRNA in liver is mRNAalb. A two-fold increase in mRNAalb production was observed after treatment of the animals with TAA. Sucrose density gradient fractionation of mRNA from TAA-treated liver, coupled with the immunoprecipitation assay, enabled us to prepare mRNAalb in reasonably pure form. C-DNA to this message has been synthesized *in vitro* using AMV reverse transcriptase. We are now in the process of preparing C-DNAalb in pure form.

F. Idenification of a Transformation-Specific Protein Induced by a Rous Sarcoma Virus

Using antisera obtained from rats bearing Schmidt-Ruppin strain Rous sarcoma virus-induced tumors, we have identified a protein with an apparent molecular weight of 56,000 daltons and an isoelectric point of 6.3 in extracts of chick embryo fibroblasts transformed by a wild-type nondefective Rous sarcoma virus (Schmidt-Ruppin strain). This protein was not found in cells infected by transformation-defective mutants with either a partial or complete deletion of the src gene, nor in cells infected by a nontransforming avian leukosis virus. The 56,000 dalton molecular weight protein was found to be synthesized at both the permissive and nonpermissive temperatures in cells infected by either of two conditional-lethal mutants that are temperature-sensitive in cell transformation. The amount of this protein, however, accumulated in cells infected by these temperature-sensitive mutants, relative to the structural polypeptides, differed significantly from that seen with the nondefective virus. Pulse-chase experiments indicate that the protein is extremely unstable, with a half-life of about 20 minutes, and does not serve as a precursor to any of the detectable virion polypeptides. Furthermore, incubation of the rat antiserum with purified, disrupted virus did not affect its immunoreactivity to this particular protein. We conclude that this 56,000 dalton molecular weight protein is a nonstructural protein specific to cells transformed by Rous sarcoma virus.

G. Biosynthesis, Immunological Specificity, and Intracellular Distribution of the Simian Virus 40-Specific Protein Induced by the Nondefective Hybrid Ad2$^+$ND$_1$

Ad2$^+$ND$_1$, a nondefective hybrid virus containing a segment of the early region of simian virus 40 (SV40) DNA covalently inserted into the human adenovirus 2 genome, enhances the growth of human adenoviruses in simian cells and induces the SV40 U antigen. This hybrid previously has been shown to code for a 28,000 (28K) molecular weight protein not present in wild-type adenovirus 2-infected cells. By radioimmunoprecipitation using sera from hamsters bearing SV40 specific tumors, we have established that the Ad2$^+$ND$_1$-induced 28K protein is SV40-specific. This Ad2$^+$ND$_1$-induced protein is synthesized as a 30 K molecular weight precursor, which is detectable only when infected cells are pulse-labeled in the presence of the protease inhibitor tosylamino phenylethyl chloromethyl ketone. Upon fractionation of labeled cell extracts, about 80% of the 28K protein is found in the plasma membrane fraction, whereas the remaining 20% is associated with the outer nuclear membrane. This protein is not detectable either in the nucleus or in the cytoplasm. Blockage of proteolytic cleavage by tosylamino phenylethyl chloromethyl ketone did not alter the topographic distribution of this SV40-specific protein, although the amount of the precursor protein in the outer nuclear membrane increased fourfold while that in the plasma membrane was proportionately decreased. This result suggests that the 28K protein is transferred from the outer nuclear membrane to the plasma membrane after posttranslational cleavage of the 30K precursor polypeptide. These data offer further support to the proposal that the 28K protein contains the determinants for SV40 U antigen and is responsible for SV40 enhancement of adenovirus growth in simian cells.

H. Tumor-Specific Transplantation antigen: Use of the Ad2$^+$ND$_1$ Hybrid Virus to Identify the Protein Responsible for Simian Virus 40 Tumor Rejection and its Genetic Origin

Cells transformed by simian virus 40 (SV40) possess a tumor-specific transplantation antigen (TSTA) that has the property of immunizing animals against syngeneic tumor challenge. We find that the early SV40 DNA segment present in the human adenovirus 2 (Ad2)-SV40 hybrid, Ad2$^+$ND$_1$, is sufficient to induce this SV40-specific TSTA in BALB/c mice. Moreover, studies on the intracellular distribution of TSTA activity in Ad2$^+$ND$_1$-infected cells, as determined by the ability of various subcellular fractions to immunize mice against syngeneic tumor challenge, have suggested a correlation between this biological activity and the presence of the SV40-specific 28,000 M_r protein coded by this hybrid virus. Both the TSTA activity and the 28,000 M_r protein are found in the plasma membrane fraction and in the perinuclear region of infected cells but are virtually undetectable in the cytoplasmic fraction. Using a hamster antitumor antiserum that can specifically immunoprecipitate the 28,000 M_r protein, we are able to demonstrate a loss of TSTA activity concomitant with the removal of this SV40-coded protein. Thus, it appears that antigenic determinants responsible for SV40-specific tumor rejection in mice are contained within the 28,000 M_r protein coded for by the early SV40 DNA segment that extends from 0.17 to 0.28 map unit.

I. Simian Virus 40-Specific Ribosome-Binding Proteins Induced by a Nondefective Adenovirus 2-Simian Virus 40 Hybrid

We have studied the intracellular distribution of the two simian virus 40-specific proteins, with apparent molecular weights of 56,000 and 42,000, detectable in human KB cells infected by a nondefective adenovirus 2-simian virus 40 hybrid, Ad2$^+$ND$_2$. After a 20-min pulse of [^{35}S]methionine, about two-thirds of the newly synthesized 56K protein and one-third of the 42K protein were found localized on the plasma membrane. The remainder of each protein was found in the cytoplasm, whereas the nuclear fraction was virtually free of either component. A significant portion of both proteins present in the cytoplasmic fraction was complexed to the 40S ribosomal subunits and was not removed by treatment with 0.5 M KCl. Moreover, the portion that was found free in the cytoplasm could bind preferentially and quantitatively to purified 40S ribosomes *in vitro*, leading us to propose that these simian virus 40 proteins may act as translational control elements in cells.

Naturally occurring murine leukemia viruses, all of which are readily isolated from mouse cells, can now be separated into three groups on the basis of their host range, interference specificity, and virus neutralization. These groups are the (a) Ecotropic (or mouse-tropic) MuLV strains which can exogenously infect mouse cells but not cells of other species; (b) Xenotropic MuLV which cannot infect mouse cells exogenously, but can infect cells of certain heterologous species, e.g., rabbit, mink, and human; and (c) Amphotropic MuLV which exogenously infect mouse cells as well as cells from heterologous species. Utilizing a single-stranded [^{3}H] DNA probe synthesized from two xenotropic (NZB-IU-1 and BALB-1U-1) viruses and an amphotropic (1504-A) virus, we examined several inbred and wild mouse strains. Our analysis has shown that the complete genomes of all of these viruses are present in multiple copies in the DNA of all mouse strains so far tested. By hybridization of [^{3}H] DNA probes and RNAs from either purified virions or from producer cells, we have been able to characterize biochemically several naturally occurring ecotropic, xenotropic and amphotropic viruses. In general, all of these viruses share a common group of sequences; in addition, they do possess a unique group of sequences. In one ecotropic (AKR) and one amphotropic (1504-A) virus, these unique sequences represent 20% of the total viral genome. By nucleic acid hybridization techniques, including hydroxyapatite chromatography, we have been able to purify and characterize the unique sequences from ecotropic-AKR and amphotropic 1504-A.

Our studies with amphotropic viruses proved to be very significant in several respects:

a) Amphotropic viruses are isolated only from several wild colonies in California. Some colonies also harbor ecotropic and xenotropic viruses. Several attempts have been made to isolate these viruses from laboratory strains without any success, yet, as noted above, the complete genomes of these viruses are present in all laboratory strains tested thus far. Since amphotropic viruses can infect mouse cells as well as heterologous cells, such viruses presumably should include two sets of sequences, one which should provide "eco-ness" and the other "xeno-ness" to these viruses. Both of the ecotropic viruses (1504-E and 4070E) isolated from wild mice as well as the Rauscher and Moloney viruses possess 40-50% of ampho-specific sequences,i.e., 10% of the total amphotropic 1504 virus genome - presumably the region for glycoprotein. On the other hand, the newly described highly leukemogenic dual-tropic (able to grow in mouse as well as in heterologous cells) MCF (mink cell focus) viruses of AKR mice contain 50% of ecotropic AKR-specific sequences (i.e., 10% of the total AKR viral genome), 50% of xeno-specific sequences, and none of the ampho-specific sequences. Thus, in nature there exist at least two entirely different sets of sequences (eco, ampho) which presumably are serving the same function -- facilitating the growth of these viruses in mouse cells. Moreover, our studies suggest that recombination between these various viral genomes may be the necessary and sufficient event endowing murine viruses with leukemogenicity.

Effect of IUdR on Transcription of SV40 DNA

IUdR is readily incorporated into SV40 DNA, which in turn is packaged in SV40 capsids to yield infectious virus. Analysis of density differences between unsubstituted SV40 DNA and DNA substituted with IUdR (20 µg/ml) indicates that IUdR incorporation is variable, but at least one-third of the thymidine residues are replaced by IUdR. When the IUdR concentration is reduced to 10 µg/ml the effect on DNA density is less, but substituted DNA is significantly more dense than unsubstituted DNA. Form I SV40 DNA substituted with IUdR was cleaved with restriction endonucleases E.coRl (4 thymidines/site), Bam H1 (2 thymidines/site) and Hpa II (no thymidines/site), all of which cut SV40 DNA at a single site. It had been anticipated that IUdR substituted in the endonuclease cleavage site would block cleavage. However, all three enzymes convert form I SV40 DNA to form III as determined by analysis on alkaline sucrose gradients; the reasons are unclear.

Studies on infectivity and Tag induction by IUdR-substituted SV40 indicate little difference between unsubstituted SV40 and SV40 substituted with IUdR at 10 µg/ml. On the other hand, SV40 substituted with IUdR at 20 µg/ml is about 30-40 fold less infectious than unsubstituted SV40 and Tag induction is greatly reduced. Cytoplasmic RNA prepared from cells infected with unsubstituted SV40 hybridized with 50% of the SV40 L strand and 50% of the E strand. RNA transcribed from IUdR-substituted SV40 (10 µg/ml) hybridized with 83% of the SV40 L strand and 80% of the E strand. Moreover, saturation of SV40 DNA required much less RNA from cells infected with substituted SV40, indicating that the concentration of viral-specific RNA was significantly higher than that prepared from cells infected with unsubstituted SV40. RNA transcribed from IUdR-substituted SV40 DNA (20 µg/ml) hybridized with SV40 DNA to about the same extent as unsubstituted SV40; however, three times as much RNA was required to achieve saturation. Thus, it would appear that moderate substitution in SV40 DNA (10 µg/ml) increases SV40 RNA transcription both qualitatively and quantitatively, analogous to the effect of IUdR on cellular RNA transcription (see section D). On the other hand, heavily substituted SV40 DNA (20 µg/ml) is much less efficient in inducing RNA transcription. It is probable that most of the heavily substituted virions are biologically inactive.

It is possible that moderately substituted SV40 DNA could provide a model for studying the effect of IUdR on enhancement of cellular RNA synthesis. IUdR could affect transcription at three different levels: DNA (promotors), RNA (processing), and protein (mutations). Substitutions at promoter sites could interfere with binding either of RNA polymerase (inhibiting RNA synthesis) or protein regulators (enhancing RNA synthesis). Substituted DNA which is transcribed into RNA missense sequences could interfere with RNA processing and lead to longer functional RNA transcripts. Similarly, RNA transcripts containing missense sequences could be translated into proteins with altered autoregulatory functions (the early SV40 protein is autoregulatory at the transcriptional level). The available data do not permit a choice from the above (and/or other) mechanisms which would explain the role of IUdR in enhancement of transcription.

K. Interactions Between SV40 and Ad2 in Coinfected Cells

This study was initiated to demonstrate whether SV40 proteins induced by AD2$^+$ND$_4$ could initiate SV40 DNA synthesis. It was known from previous work that Ad2 inhibits SV40 DNA synthesis, but this inhibition appeared to be far from complete. Accordingly, the experimental design was to coinfect BSC-1 cells with either wild type (WT) or tsA SV40 together with either Ad2$^+$ND$_4$ or Ad2$^+$ND$_3$ (0.11 - 0.18 of the SV40 genome). The infected cells were radiolabled with ^{3}H-thymidine from 24 to 25 hours post-infection, and the viral DNA was removed from infected cells by Hirt extraction. The partially purified DNA was then analyzed for Form I SV40 DNA on alkaline sucrose and isopynic EtBr-CsCl gradients. Cells infected with SV40 tsA58 (MOI 15) at 40.5 C and coinfected with Ad2$^+$ND$_4$ (MOI 10) contained no radiolabeled form I (53S) SV40 DNA. Moreover, no form I SV40 DNA was detected in cells infected with WT SV40 and coinfected with either Ad2$^+$ND$_4$ or Ad2$^+$ND$_3$ (Ad2$^+$ND$_4$ induced SV40 Tag under the same conditions). It was evident that SV40 DNA synthesis was abrogated by coinfection with Ad. Subsequent experiments were designed to determine at what point, in the infectious cycle of Ad2$^+$ND$_3$, inhibition of SV40 DNA synthesis was initiated. Accordingly, cells were infected with WT SV40 at zero time, superinfected with Ad2$^+$ND$_3$ at 4 hour intervals after SV40 infection, and pulsed from 24-25 hours after SV40 infection. Viral DNA was extracted and analyzed as above. The results indicated that SV40 DNA synthesis was normal after 10 hours of superinfection and 90% inhibited after 13 hours of superinfection. Concurrent with the diminution of the form I SV40 peak was the appearance of a new peak at 34S (presumably Ad DNA). Similar results were obtained when WT SV40-infected cells were superinfected with Ad2 at 37 C at various times; however, the onset of Ad2 DNA synthesis and the inhibition of SV40 DNA synthesis occurs at 13 hours after superinfection. It would appear from these experiments that inhibtion of SV40 DNA synthesis by Ad2 is a late Ad2 function, perhaps similar (or identical) to Ad inhibition of host cell DNA synthesis in which Ad fiber antigen has been implicated. Attempts to demonstrate SV40 DNA synthesis in BSC-1 cells infected with SV40 tsA58 at 40.5 C, superinfected with Ad2$^+$ND$_4$, and pulsed 9-12 hours after superinfection were unsuccessful, presumably due to insufficient synthesis of functional SV40 early protein during this period.

L. EBV Strains

A new virus strain, AG876, has been characterized as a "transforming" virus which, by DNA reassociation kinetics, cannot be distinguished from P3HR1, a "nontransforming strain." This indicates that deletion of genetic material is not necessary for transforming ability, as has been previously proposed. We have proceeded to compare this virus to B95-8 - the "prototype" transforming strain - both by reassociation kinetics and by restriction endonuclease analysis. B95-8 lacks 12-15 % of the sequences present in both P3HR1 and AG876 virus strains, and on preliminary analysis it appears that each virus has a different restriction enzyme fragment pattern on gel electrophoresis. In order to study virus strains by restriction enzyme analysis when very small quantities of material are available, a new technique has been developed (GT) by means of which high resolution gel autoradiographs can be obtained using only 3000 cpm (approx. 6×10^{-2} µg) of labelled (^3H) viral DNA. This technique should prove to be important in the evaluation of other virus strains since it may be capable of demonstrating differences not apparent in reassociation kinetics analyses.

At least one additional virus strain has been isolated from a tumor cell line - spontaneously infected by EBV - of a patient with poorly differentiated lymphoma.

Additional information has been obtained with regard to the B95-8 virus. We have demonstrated by hybridization and thermal elution profiles that this viral DNA is closely associated with host cell (marmoset) DNA sequences. This marmoset DNA probably occupies the deleted part (with respect to P3HR1 and AG876) of the B95-8 DNA molecule, since it has been shown by sedimentation profiles and electron microscopy that the molecular weight of B95-8 is almost identical to that of P3HR1. The exact physical relationship of the host and viral sequences cannot be discerned, but these studies illustrate the possibility that some properties of laboratory strains of EBV may not necessarily be representative of "wild-type" strains. Comparison of the polyadenylated-RNA transcripts of these other viral strains of EBV are also in progress.

M. Differences Between EBNA Negative and EBNA Positive Cell Lines

We have been able to demonstrate clear cut differences between EBNA positive (8) and EBNA negative cell lines (10) derived from patients with various lymphomas. The cells of EB positive cell lines have greater mean electronic cell volumes (probably due to a decreased nuclear/cytoplasmic ratio), and greater ability to scatter a laser beam (narrow angle light scatter) and achieve lower peak cell density under standard culture conditions than EB negative cell lines. In addition, EB negative lines fluoresce much more brightly than EB positive lines when stained with anti-human Ig FITC, and have significantly lower levels of C3b, 7S EA, and 19S EA receptors than EB positive lines. Preliminary data concerning spontaneous conversion of tumor cell lines from EB neg. to EB pos. (presumably as a result of EBV released by normal lymphocytes when transferred to laboratory culture) suggests that EBV itself may be responsible for some of these differences, although the possibility remains that EB pos. tumors are derived from a different lymphocyte subpopulation than EB neg. tumors. Further studies may elucidate these issues. The EBV reassociation kinetic profiles of tumors and their cell line derivatives have been studied. In one case the original tumor is EBV genome negative. This patient [who had American Burkitt lymphoma (BL)] subsequently had an infectious mononucleosis syndrome followed by tumor relapse. A cell line was then established with identical cytogenetics to the tumor, but EBNA positive (the relapse tumor remained EBNA negative). This case further illustrates the minor or insignificant role EBV may play in American BL. In addition, the presence of other potentially oncogenic viruses is being tested by hybridization studies in these tumors and cell lines.

N. Derivation of Tumor Cell Lines from Normal Marrow

It is interesting that three of the EB negative cell lines were obtained from microscopically normal marrow aspirates in patients with lymphoma. Chromosomal studies support a tumor origin for these lines, confirming the general feeling that non-Hodgkin's lymphoma is frequently more widespread than is clinically apparent. Studies on EB positive cell lines have revealed that all of those so far tested (6) with low C3 receptor levels produce EBV. The relationship between EBV and complement receptor expression is intriguing and currently under investigation.

O. Purine Induced Alteration of Surface Receptors

Studies with various purine derivatives, especially methylated xanthines, have indicated that induction of C3B receptors in two cell lines (EW and CA) can be achieved by incubation with theophylline, caffeine, IBMX and aminophyline. Allopurinol also appears to induce these receptors, whereas purine and adenine inhibit theophylline induction. This induction process appears to be dependent upon new RNA and protein synthesis, but the precise mechanism has not been determined, although current evidence is not in favor of a role for cyclic AMP. Theophylline also affects Fc receptors and can inhibit complement receptors in other cell lines. The potential of these types of studies for increasing our understanding of the significance of surface receptors in lymphoma classification is currently being explored.

Significance to Biomedical Research and the Program of the Institute:

In studies on the mouse-tropic, xenotropic, and amphotropic murine leukemia viruses, our objective is to correlate specific leukemia virus sequences with specific biological events, especially leukemogenesis. Comparative study of the genetic information specified by different murine leukemia virus sequences in different strains of mice is now feasible, and we believe that it will be possible to determine the evolutionary origin and transmission of murine leukemia virus infection. Our studies during the past year have explained why only some mice produce virus and develop leukemia even though all mice may contain MLV antigens. Moreover, it has been established that a major mouse chromosomal locus for the production of MLV is in fact composed of MLV nucleotide sequences, and it is not merely a host locus which permits the expression of remote viral functions. Finally, our recent studies suggest that recombination between the genomes of various naturally occuring murine viruses may be the necessary and sufficient event which eventuates in leukemogenicity. It should be emphasized that these studies concern known naturally occuring animal leukemia viruses within the context of known and controlled host genetics. We believe that these studies will guide the framing of testable hypotheses in human leukemia, wherein a proven leukemia virus has not been characterized and where the genetics of the disease are not known and cannot be controlled.

Retroviruses are extremely efficient in transforming cells grown in culture, and these morphological transformants are bona fide tumor cells. However, the molecular mechanism leading to such transformation is not known. Our success in identifying transformation-specific proteins induced by certain RNA tumor viruses should improve our understanding of the biochemical events responsible for viral carcinogenesis. Such information may lead to the design of methods for the control of malignant conversion of normal cells by retroviruses.

Our studies of Ad2-SV40 hybrids have made a significant contribution to knowledge of genetic organization and expression of the two tumor viruses during lytic infection and oncogenesis. Moreover, these hybrid virus studies offer insight into the state and expression of integrated heterologous DNA in general and must be considered in planning the type of "genetic engineering" experiments possible with recombinant DNA technology.

Whether a specific viral infection will lead to virus production or to cell transformation depends entirely upon the interaction between the incoming virus and the host cell involved. At the molecular level, such interactions may occur between virus-specific macromolecules and macromolecules specified by the host. Our studies with Ad2 and SV40-antigens are directed toward an understanding of these interactions; our hope is to provide a better understanding of the mechanisms of viral infection and oncogenesis. Since the presence of tumor-specific transplantation antigens in viral-induced tumor cells may permit immune modulation of the growth of antigenic tumors, the biochemical characterization of such macromolecules is of potential importance. Our ability to assign the tumor rejection activity induced by SV40 to a specific polypeptide chain (28K) should permit study of the molecular mechanism of action of a single macromolecule in this regard.

EBV is closely associated with at least three human diseases - infectious mononucleosis, African Burkitt's lymphoma and nasopharyngeal carcinoma. As such it represents a potential human tumor virus, and clarification of its role in the diseases with which it is associated is of paramount importance. Studies on the induction of EBV with corticosteroids may clarify the relationship of EBV to the host cell since they suggest that the viral genome may be influenced by mechanisms which regulate the transcription of unique genes in differentiated cells. Such information is pertinent to an understanding of the association between latent virus infection and oncogenesis - both of which are important characteristics of the herpes virus group as a whole. Studies of different strains of EBV will help to determine whether or not differences in virus properties are relevant to the association of EBV with sub-clinical, benign and malignant diseases. Finally, our use of better defined reagents is revealing differences in the surface receptors of lymphoma cells which were previously not realized, and which may be important in more precisely defining the counterpart normal cell. Hopefully these findings will provide an improvement in the classification of lymphomas as well as permitting more selective therapy.

Proposed Course:

We shall continue to employ various biochemical and biological techniques to distinguish between the viral sequences specified by different endogenous murine leukemia viruses in the DNA of mice with differing expression of MLV information. DNA from other rodent species will be tested for homology with various MLV DNA probes, and these studies should speak to the evolutionary origin, transmission, completeness and expression of the MLV genome. We shall attempt to delineate further the molecular mechanism of viral activation by halogenated pyrimidines, and will also extend these studies with other activating agents.

Studies employing the nondefective Ad2-SV40 hybrid viruses will emphasize the antigenic relationships between each of the early SV40-specific proteins and attempt to identify the antigenic determinants and molecular structure responsible for each of the SV40 immunological and functional activities. It has been suggested that SV40 T antigen may have a pleiotropic effect on various cellular functions in viral transformation. Since each of the Ad2-SV40 hybrid viruses has been shown to synthesize SV40-specific polypeptides of different molecular weights but with overlapping amino acid sequences, we shall attempt to use the hybrids to map the "active sites" of various cellular functions, especially transformation, and the various properties of T antigen (which appears to be coded by the entire early region of the SV40 genome).

With respect to wild-type adenovirus and SV40, we shall further explore their interactions in doubly infected cells, particularly Ad inhibition of SV40 early functions. We shall use fragments of SV40 (and hybrid) DNA to complement SV40 temperature-sensitive mutants at nonpermissive temperatures. If this approach is successful, it should be possible to superinfect with Ad2 DNA fragments and determine which part(s) of the Ad genome inhibit early SV40 functions. If specific Ad2 DNA fragments can be demonstrated to block early SV40 functions, it will be of great interest to see whether they interfere with SV40 transformation.

In our experiments with Epstein-Barr virus, we shall continue to optimize the system for production and purification of this virus and its DNA: we shall measure the genetic relatedness of EBV strains of different origin, study the relationship between EBV DNA and host cell DNA, and try to relate our findings to EBV-associated human disease. Further studies are planned of the mechanism of steroid induction of EBV - e.g., the role of steroid receptors. It is hoped that more virus strains can be obtained, and comparisons made with the viral sequences present in cell lines derived from patients with infectious mononucleosis, and from throat-washing induced cell lines obtained from patients in different parts of the world. These studies will be extended by utilizing restriction endonuclease fragmentation of the viral genomes. We shall also continue in our efforts to determine whether the induction of receptors on lymphoma cells is brought about by direct switching on of a unique gene, representing a differentiation step, and whether or not cyclic AMP is involved. In addition we shall attempt to develop reagents which give a much greater degree of precision in identification of surface receptors. These studies will be extended by examining other functional characteristics of tumor cells or their derived lines and correlating such a functional classification of lymphoma with morphology and clinical course.

Through the use of our albumin cDNA probe, the levels of mRNAalb can be measured precisely in normal, precancerous and malignant tissue. Our studies employing TAA, a liver carcinogen, indicate that an increase in the level of mRNAalb may be associated with the onset of hepatoma. We shall pursue this observation to determine its biological implications, and the value of mRNAalb as a tumor marker.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06860-02 PO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Clinical and Molecular Pharmacology

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LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	4	2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project concerns clinical, preclinical and biophysical studies of anticancer drugs. A clinically useful assay for citrovorum factor and 5'methyltetrahydrafolic acid has been developed and applied to clinical situations. Preclinical studies utilizing a primate model have also been done using this assay. Other preclinical studies of systemic and cerebrosoinal pharmacokinetics have been initiated using a variety of anti-neoplastic drugs. Drug-drug interactions have also been explored using this model. The alkaline elution technique (Kohn and Ewing, Biochemistry 1974) has been employed in a study of the interaction of intercalating agents with nucleic acids in vitro (i.e., cells). Such intercalating agents have included adriamycin, daunomycin, eliptocine and actinomycin D. Light-sensitive photoreactions with DNA by certain intercalating agents, the psoralens, have also been studied using this technique. Physico-chemical measurements, including electric dichroism and hydrodynamic studies, have also been initiated on these compounds and their derivatives. The object of these studies is to be able to employ such compounds as therapeutic agents and to utilize compounds such as these, with known mechanisms of actions, as probes in examining the mechanism of action of other chemotherapeutic agents whose mechanisms are not defined.

Objectives:

1. To develop and apply clinically useful assays for chemotherapeutic agents.

2. To characterize the pharmacokinetics of chemotherapeutic agents and related compounds and thereby to enhance their utility.

3. To utilize physical-chemical techniques to characterize the complexes of small molecules (both antineoplastic agents and carcinogens) with nucleic acids and other biopolymers.

4. To utilize the information gained in #3 to facilitate evaluation of analogs of known chemotherapeutic agents.

5. To utilize agents characterized in #3 and #4 as probes of macromolecular and subcellular structure in neoplastic cells.

6. To improve the therapy of meningeal leukemia by detailed investigation of cerebrospinal fluid pharmacokinetics in a primate model.

7. To evaluate the pharmacokinetics of chemotherapeutic agents used to treat central nervous system malignancies.

Major Findings and Methods

A. An assay system for citrovorum factor (N-5-formyl tetrahydrofolic acid) has been developed using a bacterially derived cell-free synthesizing system with appropriately radiolabeled components. The system appears to be sensitive to approximately 10^{-7} molar citrovorum factor. Using this assay system, citrovorum factor disappearance curves and 5-methyltetrahydrafolic acid disappearance curves have been measured in a series of patients and in the primate model following intravenous bolus injections and infusions. Results indicate that the half-disappearance time of citrovorum factor is significantly shorter than that of methotrexate and in fact approximates 15 minutes as compared to a 2 hour disappearance time for methotrexate itself. The 5'methyl derivative (5'methyltetrahydrofolic acid) has a much longer half-disappearance time, but the conversion of the citrovorum factor to the methyl derivative appears to be rather inefficient, with approximately 10% of the dose converted and the maximum conversion being achieved 1 hour after an intravenous bolus injection of citrovorum factor. Preliminary data indicate that the cross-over into the central nervous system of the methyl derivative is much more efficient that that of the citrovorum factor itself following an intravenous injection (by about a factor of 100). We are presently measuring the pharmacokinetics following intrathecal administration of these compounds.

B. We have continued our interest in the compound 8-azaguanine, using a spectroscopic assay which was developed last year. Clinical data is presently being accumulated in collaboration with Dr. Ayoub K. Ommaya, on patients with gliomas.

C. We have extended studies of the effect of systemic probenecid on intrathecal administration of methotrexate in the primate model; the results obtained are similar to those previously observed in the dog. Observations in patients, both at this institution and the University of Washington, indicate that there is a component of active transfer of methotrexate from cerebrospinal fluid to blood; these observations should allow application of the results of animal studies to clinical situations. Data have also been obtained on the influence of pretreatment with vincristine on methotrexate pharmacokinetics in monkeys, using both low and high dose methotrexate infusions. A marked alteration in methotrexate pharmacokinetics was observed and is presently being evaluated. Data on other compounds of therapeutic interest, in particular the nitrosoureas, are also being obtained.

D. We have applied the alkaline elution technique, in collaboration with Drs. Kurt Kohn and Warren Ross, to the study of interactions between intercalating agents and whole cells (in vitro). Following the binding of a variety of intercalating agents, there appears to be formation of protein complexes at the site of DNA breaks as well as the formation of protein-DNA cross-links. Both of these phenomena appear to occur in approximately equal number following exposure to intercalating agents. Further studies to characterize the protein involved, as well as the nature of the DNA breaks and their repair mechanism, are underway. Interactions of psoralens with whole cells have also been studied using the same technique. The psoralens, in reactions with naked DNA, exhibit light-sensitive DNA-DNA cross-linking effects with essentially no protein binding. Similar results have been obtained in whole cells and these results are being evaluated with respect to their biological significance. Moreover, results with the psoralens suggest their possible utility as chemotherapeutic agents. These compounds are also being used in conjuntion with studies of mechanism of action of other chemotherapeutic agents. Biophysical studies are proceeding, including electric dichroism measurements of the psoralen complexes with nucleic acid, and of complexes with other intercalating agents (in particular, adriamycin derivatives).

Significance to Biomedical Research and the Program of the Institute

The availability of a clinically applicable assay for rescue agents employed in the administration of antifolates will allow characterization of these rescue agents in various body compartments under a variety of therapeutic circumstances. The citrovorum assay may permit the utilization of antifolate agents which previously had unacceptable toxicity, since new knowledge of the pharmacokinetics of these antifolates (which differ significantly from methotrexate) may permit the design of appropriate rescue regimens. This assay will also permit one to assess the relative rescue effect of folic acid derivatives in various clinical settings, in particular the use of systemic antifolates in the treatment of central nervous system or other sanctuary disease. Studies of the interaction of antifolates, especially methotrexate, with other compounds will permit more rational utilization of such combinations. Studies on the pharmacokinetics of other chemotherapeutic agents, e.g., the nitrosoureas, will have direct clinical applicability upon completion. Basic biological and biophysical studies on the interaction of chemotherapeutic agents with whole cells and with biologically important macromolecules should enhance the utility of these agents clinically. Such studies should also prove useful in attempts to solve basic biochemical problems related to the process of neoplasia. Finally, the development of an animal model allowing repetitive sampling of ventricular cerebrospinal fluid is highly significant because the model permits accurate pharmacokinetic analysis of antineoplastic agents in CSF and systematic investigation of agents having potential efficacy in central nervous system neoplasia of either leukemic or non-leukemic origin.

Proposed Course:

As outlined above, it is our intention to utilize those assay systems which are clinically applicable at the present time to explore a fairly wide range of problems related to antifolates, including distribution of rescue agents and the design of rescue regimens for those antifolate compounds which are presently thought to be of limited utility. We are also developing new assays for important drugs such as 8-azaguanine. We intend to extend our studies on the interaction of methotrexate with drugs such as probenecid to man. Molecular pharmacology studies on intercalating agents (e.g., the psoralens) will continue with the hope that these interesting agents can be applied to the treatment of human neoplasia (e.g., mycosis fungoides).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06870-02-PO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Regulation of Differentiation in Normal and Leukemic Hematopoietic Cells.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	J. Greenberger, Assistant Professor, Sidney Farber Center	Harvard Univ.

COOPERATING UNITS (if any)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The techniques of cell and molecular biology are used to analyze mechanisms which control the expression of differentiated genes in normal and leukemic hematopoietic cells. A chromosomal-dependent APRT-specific method of interspecies transfer of the human alpha globin gene (HAGG) has been developed. Using this method, we have isolated mouse erythroleukemia hybrid cells in which retention at high or low frequency of the HAGG occurs. Full expression and stable retention of this gene are observed when the human donor cell is of erythroid origin. Transcription of the HAGG without translation to globin chains occurs when the donor cell is a human peripheral blood leukocyte. We are attempting to elucidate the mechanism of this control as well as to identify other types of regulation. Study of a hybrid derived by fusion of a rodent erythroleukemia cell with a human promyelocytic leukemia cell has led to the conclusion that maintenance of myeloid differentiation in the hybrids is dependent on the presence of a ploidy excess of chromosomes of myeloid origin. Such hybrids are being studied to clarify further the defect in human leukemic hematopoietic cells which leads to the accumulation of immature but committed myeloid precursor cells (blasts).

PHS-6040
(Rev. 10-76)

Objectives:

1. To study the mechanism(s) regulating the expression of one marker of erythroid differentiation, globin gene expression, as a model for the regulation of expression of markers of myeloid and erythroid differentiation in human normal and leukemic states.

2. To establish continuously proliferating, genetically stable cell lines of human and rodent origin in which the expression of markers of myeloid differentiation occurs, in order to identify the genetic mechanism(s) which control the expression of these markers.

3. To transfer the structural genes coding for the markers of myeloid and erythroid differentiation, using chromosome-dependent methods, from normal and leukemic human cells to rodent leukemia cells in which expression of these human phenotypes occurs. These gene transfer experiments may permit the identification of genetic loci (syntenic to the structural genes) which govern the expression of the markers of myeloid and erythroid differentiation, and may also permit the identification of mechanisms by which hematopoietic cells become malignant.

4. To utilize knowledge of the mechanisms which lead to the orderly differentiation of hematopoietic cells so as to design genetic or other strategies for correcting defects in the process of differentiation and cell renewal as observed in leukemia.

5. To purify substances which may regulate expression of markers as well as structural genes in normal hematopoietic cells, and to compare such substances from normal and leukemic populations.

Methods Employed:

The techniques of cell biology (cell culture and cloning, chromosomal analysis by light and fluorescent microscopy, mutagenesis, isozymal analysis, radioisotopic analysis of enzyme activity and protein synthesis, cell fusion, and development of *in vitro* systems for the study of gene expression) are combined with those of molecular biology (molecular hybridization assays for the presence and expression of unique gene sequences, oligo dT chromatography of mRNA, hydroxylapatite chromatography of DNA, synthesis of cDNA corresponding to the globin mRNAs of man and mouse, density gradient ultracentrifugation, gel electrophoretic separation of nucleic acids, restriction endonuclease mapping, R-loop mapping, cell-free systems for the translation of putative mRNA populations from hybrid cells, isoelectric focusing of proteins) in order to study mechanisms governing regulation of gene expression in mammalian cells. A tissue culture laboratory equipped with environmental control devices is employed, as well as a complete laboratory for the study of gene expression at the molecular level.

Major Findings:

A. Development of a chromosomal-dependent APRT-dependent method of interspecific transfer of the human alpha globin gene:

We have demonstrated that introduction of human chromosomes from erythroid cells bearing the globin structural genes into mouse erythroid cells (MEL) by cell fusion generates cell hybrids in which the co-expression of human and mouse globin genes is observed. In contrast, fusion of the MEL cell with human fibroblasts results in cells in which failure of expression of the human globin genes as well as loss of expression of the mouse globin genes has occurred at the level of transcription. Furthermore, dimethyl sulfoxide, which stimulates the transcription of the globin genes in the MEL cell but has no effect on the expression of human globin genes in the normal human erythroid cell, stimulates the expression of the human globin genes once they have been introduced into the MEL cell. Since the globin genes of different species on different chromosomes in the same cell respond to the same exogenous stimulus, some workers have concluded that the induction process involves diffusable regulatory substances. These hybrid cells therefore would appear to have been a good system for the study of the gene expression were it not for the loss of human chromosomes which occurs from these cells during continued culture *in vitro*.

We have recently developed a method of transfer of the human alpha globin gene to rodent erythroleukemia cells in which stable retention and expression of the human alpha globin gene (HAGG) is observed. This system is based on the use of a mouse erythroleukemia cell (MEL) which is deficient in the enzyme adenine phosphoribosyl transferase (APRT), a marker known to be present on human chromosome (HC) 16 (to which we have recently assigned the HAGG). We fused this APRT-deficient MEL cell to populations of human marrow enriched in erythroid cells, in the presence of Sendai virus. Isolation of hybrid cells in the AAT selective system (adenine, thymidine and aminopterin) promotes the growth of hybrid MEL cells which have retained HC 16, and kills all of the unfused MEL parent cells which cannot utilize exogenous sources of adenine because of the APRT deficiency (and which depend on adenine due to the presence of aminopterin). The hybrid cells thus isolated were dominated by cells which had retained HC 16 but very few other human chromosomes. These hybrid cells also exhibited stable retention of HC 16 for several months, and well as the stable expression of the HAGG both at the level of transcription and translation. Thus, this APRT-specific chromosomal-dependent system of transfer of the HAGG results in hybrid cells in which stable retention and continuous expression of the HAGG occurs.

B. Use of the APRT-specific, chromosomal-dependent method of transfer of the HAGG in combination with diaminopurine counterselection to isolate cell lines with high and low frequency of the HAGG and to confirm the assignment of the HAGG to HC 16:

2,6-diaminopurine, a compound lethal to cells in which it is incorporated, was used to select for the human marrow X MEL hybrid cell population described above (from which loss of the HAGG and HC 16 had occurred). Thus, cells from which loss of HC 16 has occurred become resistant to the action of the substituted purine analogue. Populations of the human marrow X MEL hybrid cells were exposed to increasing concentrations of diaminopurine, and the cells which emerged in this selective system were characterized with respect to their chromosomal composition as well as their pattern of expression of globin genes. The frequency of human chromosome 16 was observed to drop from 80% to 0% in the hybrid populations after diaminopurine selection. In contrast to the human marrow X MEL hybrid cell before diaminopurine selection (in which substantial quantities of human alpha globin mRNA had been identified), the diaminopurine-selected cells contained no detectable levels of human alpha globin mRNA. Thus, the APRT-specific chromosomal-dependent method of transfer of the HAGG led to hybrid cell populations in which the high or low frequency of the HAGG (and HC 16) correlated perfectly with the expression of the HAGG. These studies confirmed our earlier assignment of the HAGG to HC 16 which had been based on study of hybrid cells derived by fusion of mouse and human fibroblasts. In the earlier studies, no opportunity to study expression of the human globin genes existed.

C. Use of the APRT-specific, chromosomal-dependent method of transfer of the HAGG to assess the potential for expression of the HAGG donated by cells of erythroid and non-erythroid origin.

In order to test if the expression of the HAGG depended not only upon the presence of the HC 16 but also upon the origin of the donor cell of HC 16, we isolated hybrid cells by fusion of the APRT-deficient MEL cell with populations of human peripheral leukocytes which had been depleted of identifiable nucleated erythroid cells (using ficoll-hypaque gradient centrifugation). Following isolation of hybrid cells in AAT selective medium, which ensures retention of HC 16, we studied the chromosomal composition and the pattern of expression of human globin genes in this hybrid cell. 60-70% of the cells of this hybrid population contained HC 16 even after several months of continuous culture. In contrast, there were no detectable human alpha globin chains in these hybrid cells even after dimethyl sulfoxide induction of expression of the globin genes, and in spite of continued expression of the mouse beta and alpha globin genes. Thus, the presence of HC 16 is necessary but not sufficient for the expression of the HAGG, which also depends upon the origin of the chromosome. Hybridization of the cytoplasmic RNA of the human peripheral blood leukocyte X MEL cells with human alpha globin cDNA revealed the presence of human alpha globin coding sequences in amounts lower than those found in the human marrow X MEL cells. The presence of human alpha globin coding sequences without the accumulation of human alpha globin chains suggested that a portion of the human globin coding sequences had been deleted in the hybrid cells. As a test for this possible explanation of the results, increasing quantities of polyadenylated cytoplasmic RNA from the hybrid cells were incubated with human alpha globin cDNA in a hybridization assay. We wished to see if the globin coding sequences in the RNA of the hybrid cells could anneal and protect the human alpha globin cDNA to the same extent as can its template, human alpha globin mRNA. The polyadenylated cytoplasmic RNA of both the human marrow X MEL and the human leukocyte X MEL hybrid cells protected the human alpha globin cDNA from the action of single-strand specific nuclease to a degree which was equal to that observed with human alpha globin mRNA. 330 and 1000 times as much of the polyadenylated cytoplasmic RNA was required of the human marrow X MEL and the human leukocyte X MEL hybrid cells, respectively, than was needed when using purified human alpha globin mRNA. This result reflects the lower concentrations of the human alpha globin coding sequences found in these hybrid cells.

Thus, this system permits the study of mechanisms which govern expression of the human globin genes; such regulating genes appear to be carried with the chromosome bearing the structural genes when the latter are transferred to rodent leukemia cells using the gene transfer system.

D. Application of fluorescent-activated particle cell sorting for the isolation of hybrid cells containing high or low levels of the human chromosome 11 in order to test the validity of our recent assignment of the human beta and gamma globin genes to H.C. 11:

Antisera specific for human x mouse hybrid cells which contain human chromosome 11 were developed by Dr. Burt Dorman of Yale University. Populations of hybrid cells, which we had shown in previous studies to contain low but detectable levels of both human chromosome 11 and the human beta globin gene, were subjected to automated particle cell sorting using chromosome 11-specific fluorescent antiserum and the Bectin-Dickinson particle cell sorter (Dr. Frank Ruddle, Yale). Cell lines with varying levels of H.C. 11 were identified by chromosomal analysis. These cell lines are currently under study.

E. Isolation of leukemic hybrid cells (derived by fusion of rodent promyelocytic leukemia cells with mouse erythroleukemia cells) which exhibit expression of markers specific for myeloid but not erythroid differentiation

In order to characterize the genetic mechanism governing myeloid differentiation in normal and leukemic cells of rodent and human origin, we have isolated hybrid cells derived by fusion of 6-thioguanine-resistant tetraploid rat promyelocytic leukemia cells (which exhibit constitutive production of alkaline phosphatase, specific esterase and myeloperoxidase) with diploid mouse erythroleukemia cells (which produce hemoglobin when incubated in the presence of dimethyl sulfoxide). Study of populations of these hybrid cells with a fluorescent stain specific for rat and mouse chromosomes (Hoechst 33258) confirmed that a ploidy excess of the chromosomes derived from the myeloid parent was retained by the hybrid cells. One hundred per cent of these hybrid cells produced all three enzymes (which serve as markers for myeloid differentiation in the parent rat promyelocytic leukemia cell) but did not produce hemoglobin despite retention of the chromosome bearing the structural gene for mouse beta globin. These experiments suggest a relationship between the numbers of chromosomes of myeloid and erythroid origin retained and the expression of genes which serve as markers for myeloid and erythroid differentiation in normal and leukemic hematopoietic cells. Strains of the parent promyelocytic leukemia cell and the hybrid cells suggested, however, that the hybrid cells may be unable to package the myeloid enzymes into intracellular granules as do normal myeloid cells. Electron microscopic studies with reagents which can detect peroxidase at the ultrastructural level are being used to clarify this issue.

Significance to Biomedical Research and the Program of the Institute:

Our objective has been to clarify the genetic and molecular mechanisms which control the orderly division and maturation of hematopoietic cells. Virtually all leukemic states involve a breakdown in the orderly maturation of each type of differentiated cell, or the complete arrest of the ability of these cells to mature normally, resulting in the accumulation of immature committed precursor cells and the cessation of production of the normal myeloid, erythroid, or lymphoid cells necessary for the survival of the patient. Clarification of the genetic mechanisms underlying these control processes in normal hematopoietic differentiation, as well as identification of the defective locus of control in the transformed state, may contribute to our ability to understand and effectively treat these disorders.

Proposed Course:

We are applying our system of interspecific transfer of the HAGG to the study of hybrid cells derived by fusion of the APRT-deficient MEL cell with populations of human fibroblasts, using both diploid and tetraploid MEL cells. We are also examining the human alpha globin coding sequences from the human leukocyte X MEL hybrid cells to see if they serve as suitable messengers in cell-free translational systems. We shall employ formamide gel electrophoresis, as well as R-loop mapping and restriction endonuclease analysis, to determine if the presence of additional non-globin coding sequences occurs in the transcripts containing human alpha globin coding sequences as they are found in the human leukocyte X MEL hybrid cells. Such aberrant transcription could possibly account for the absence of translation of these human alpha globin coding sequences into detectable levels of human alpha globin chains. Finally, the human leukocyte X MEL hybrid cells, as well as the hybrid cells derived by fusion of human fibroblasts with MEL cells, will be used to attempt to identify and purify both the structural genes for the markers of erythroid differentiation as well as the putative regulatory substances which govern their expression. Such studies should permit one to assess whether leukemic cells differ from normal hematopoietic cells in the patterns of differentiated gene expression and/or in the substances which govern this expression. The fusion system will also be used to study genetic determinants of myeloid differentiation. The studies which have resulted in the isolation of hybrid cells derived by fusion of rodent erythroid with myeloid cells will be continued in order to isolate hybrid cells in which a different balance of erythroid and myeloid chromosomes will alter the pattern of myeloid and erythroid phenotypes. Functional assays (bactericidal function, phagocytosis, chemotaxis) will also be applied to the study of these hybrid cells as well as to others yet to be isolated to see if these more complex differentiated myeloid functions are governed by the same mechanisms as observed for the expression of alkaline phosphatase, peroxidase and specific esterase. A human myelogenous leukemia cell line will be subjected to similar methods of analysis in order to ascertain if the expression of markers of myeloid differentiation is subject to regulation by mechanisms which were observed in the leukemic hybrid cells of rodent origin.

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3. Deisseroth, A.B., and Hendrick, D.: Human alpha gene expression following chromosomal dependent gene transfer into mouse erythroleukemia cells. Cell, in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06880-01 PO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Experimental Approaches to the Treatment of CNS Malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. G. Poplack, Senior Investigator	PO C
Other:	D.L Glaubiger, Senior Investigator	PO C
	D. Gangji, Guest Worker	PO C
	W. A. Bleyer, Assoc. Prof. of Ped.	U. of Washington
	J. Wood, Asst. Prof. of Neurosurg.	U. of Pennsylvania
	A. Ommaya, Acting Chief, Surg. Neurol. Br.	SN N
	G. Dichiro, Chief, Neuroradiology	SN N
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	J. M. Strong, Senior Investigator	LCHPH C

COOPERATING UNITS (if any) Surgical Neurology Branch (NINCDS); Biological Psychiatry Branch (NIMH); Lab. of Clinical Science (NIMH); Radiation Oncology Branch (NCI); Lab of Chem. Pharm. (NCI); University of Washington.; Univ. of Penn; Walter Reed Med. Center; Johns Hopkins Univ.

LAB/BRANCH
Pediatric Oncology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

TOTAL MANYEARS: 6	PROFESSIONAL: 4	OTHER: 2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Experimental approaches to the treatment of meningeal leukemia and other meningeal and non-meningeal CNS neoplasms are explored. A unique subhuman primate model which allows sterile, repetitive access to the cerebrospinal fluid is utilized to study the CNS pharmacokinetics of various intrathecally and intravenously administered chemotherapeutic agents, to evaluate the neurotoxicities attendant upon various chemotherapeutic and radiotherapeutic treatments; and to evaluate and screen, in a preclinical setting, newer CNS treatment modalities and drug schedules. Information gained from studies with this model is then applied to the design of clinical protocols used to treat patients with meningeal and non-meningeal CNS malignancies.

PHS-6040
(Rev. 10-76)

Objectives:

1. To develop a subhuman primate model which provides repetitive access to the cerebrospinal fluid and allows detailed study of the pharmacology and neurotoxicity of chemotherapeutic agents used to treat CNS malignancy.

2. To study the CNS pharmacokinetics of currently useful and potentially useful CNS antineoplastic agents.

3. To assess the neurotoxicity of chemotherapeutic agents used in the treatment of CNS malignancy.

4. To better understand the physiology of the blood brain barrier, using the subhuman primate model.

5. To utilize neurophysiological and neuropharmacologic information gained in the experimental primate system as a basis for designing new clinical approaches to the treatment of CNS malignancy in man.

Methods Employed and Major Findings

A. Development of a subhuman primate model

We have developed an experimental subhuman primate model which enables repetitive, sterile sampling of ventricular cerebrospinal fluid (CSF) over extended periods of time in unanesthetized rhesus monkeys. In this model, the chamber of an Ommaya reservoir is implanted subcutaneously over the occiput in rhesus monkeys. The tip of a silastic Pudenz catheter is passed through the foramen of Magendie into the fourth ventricle, its opposite end connected to the reservoir chamber. The development of this model has provided a means of evaluating the CNS pharmacokinetics of antineoplastic agents. Pharmacokinetic studies utilizing this experimental system have proven its validity as a substitute for experimentation in the human situation. Human and primate data are strikingly similar, indicating that the kinetics (and presumably the mechanisms) of transport between the CSF and plasma compartments are similar in man and monkey.

B. Pharmacokinetic Studies

1. Methotrexate - Initially the primate model was used to study the CNS pharmacokinetics of methotrexate. Methotrexate concentrations were measured utilizing the dihydrofolate reductase inhibition assay. Following intra-CSF administration, the disappearance curves of methotrexate from CSF and plasma are similar to those obtained in man given comparable doses. During an intravenous infusion, the CSF: plasma ratio obtained in the monkey is similar to that in man. Methotrexate injected as an intravenous bolus peaks in the CSF at 0.5 to 1 hour following administration and declines in a biphasic manner. The ability to achieve therapeutic concentrations of MTX in the CSF

following intravenous injection is now being explored as a possible clinical approach to CNS malignancy in man (see below).

2. Methotrexate analogs

The CNS pharmacokinetics of a number of different antifolate compounds are being assessed in the primate system. Studies with aminopterin have been of particular interest. This compound is more potent than methotrexate in that 1/5th to 1/10th of the dose results in the same antitumor effect. In contrast, the concentrations causing acute neurotoxicity appear to be the same for both drugs. We have demonstrated that following an intraventricular injection, aminopterin disappears from the CSF considerably more slowly than methotrexate. Between 48-96 hours following injection of aminopterin (at 1/5th the dose of methotrexate), several-fold higher levels of aminopterin are present in the CSF than are achieved with methotrexate. These observations suggest that when compared to methotrexate, intrathecal aminopterin may provide more sustained antifolate levels in the CNS at one-fifth the dose, with potentially less neurotoxicity.

3. Hyperbaric approaches to CNS therapy

Hyperbaric intrathecal chemotherapy refers to the use of gravity to shift a drug containing a heavier-than-water solution within an anatomic compartment. This method is usually performed with the individual placed in the Trendelenberg position. We have evaluated the approach in the primate model and have found that when methotrexate is administered with D-10-W (a heavier-than-CSF fluid) as the diluent, substantially higher ventricular CSF levels of the antifol are achieved than when the diluent is Elliot's B solution (an artificial CSF). These results suggest that the use of the hyperbaric technique may have a clinical advantage when intrathecal chemotherapy is administered by lumbar puncture.

4. Alteration of methotrexate pharmacokinetics by probenecid

We have been interested in determining whether methotrexate pharmacokinetics can be altered in such a way as to increase the drug's therapeutic efficacy. We studied the effect of probenecid on the efflux of methotrexate from the cerebrospinal fluid in monkeys. The methotrexate-CSF disappearance curves were compared in animals pre-treated with or without intravenous probenecid. Probenecid administration resulted in considerably higher CSF methotrexate levels than those achieved with the same methotrexate dose in the absence of probenecid. The increase in CSF methotrexate concentration was achieved with only a minimal alteration in plasma methotrexate level, suggesting that methotrexate is removed from the CSF (at least in part) by an active transport mechanism.

5. Citrovorum Factor

The availability of a sensitive assay for citrovorum factor developed by Drs. E. Hamel and D. Glaubiger has allowed us to embark on studies designed to elucidate the pharmacokinetics of citrovorum factor. These studies are in progress.

6. Radiosensitizers

Considerable interest has developed in the use of certain compounds with radiosensitizing properties as potential adjuncts to the radiotherapeutic management of CNS neoplasms. We are currently studying the pharmacokinetics of these compounds in an effort to provide information necessary for rational dose scheduling of these agents (in collaboration with Drs. J. Strong and R. Adamson).

7. Corticosteroids in the CNS

We have studied the CNS pharmacokinetics of the three most commonly utilized corticosteroid compounds, dexamethasone, prednisolone and hydrocortisone. These agents are used to treat the acute symptoms associated with cerebral edema, and in the treatment of meningeal and non-meningeal CNS neoplasms. We have shown that following an intravenous injection, hydrocortisone enters the CSF more rapidly than the other compounds. However, dexamethasone, in addition to having the highest glucocorticoid potency of these steroids, has a longer CSF and plasma half-life. These studies offer a pharmacokinetic basis for the preferred use of dexamethasone in the treatment of CNS malignancy and its complications.

8. Combination Chemotherapy

Studies have been performed to assess the effect of drug-drug interactions on the CNS pharmacokinetics of intrathecally administered agents. Hydrocortisone, cytosine arabinoside, and methotrexate are used together clinically for intrathecal treatment of meningeal leukemia. We have found that the addition of cytosine arabinoside and hydrocortisone to intrathecal methotrexate has no adverse effect on the disappearance kinetics of MTX from the CSF. Thus, the recently observed lack of an additive clinical effect using these three agents in combination cannot be explained on a pharmacokinetic basis.

Studies are also in progress to evaluate the CNS pharmacology of adenine arabinoside (ARA-A) plus deoxycoformycin. This combination has proven to be effective in the treatment of meningeal leukemia in animals and is being considered for clinical trials in humans.

9. Other Agents

A variety of other chemotherapeutic compounds are being screened and evaluated in the primate model to determine their ability to penetrate the CNS. We have found, for example, that following intravenous injection of cis-platinum diaminodichloride (DDP), substantial crossover of this compound into the CSF occurs.

C. Neurotoxicity

1. Animal studies

The primate model has been used to assess various forms of neurotoxicity. Recently, our major interest has been in the development of a model of methotrexate leukoencephalopathy. In a pilot study, monkeys were given 3500 rads of cranial irradiation, and a continuous CSF methotrexate level greater than 10^{-8} molar was maintained in these animals by biweekly intraventricular injections. Within 4 months, the animals became profoundly neurotoxic and expired. At autopsy, histological examination of their brains revealed lesions identical to those seen in patients who have developed diffuse necrotizing leukoencephalopathy following cranial irradiation and either intrathecal or intravenous methotrexate therapy. Thus, in these preliminary experiments, we have been able to produce an experimental leukoencephalopathy model. In subsequent studies, we shall attempt to delineate those factors responsible for the development of this syndrome as well as to study various clinical indices that might be of value in following patients at risk.

2. Patient Studies

In addition to C-T scan monitoring of patients at risk of therapy-induced leukoencephalopathy (see report Z01-CM-06810-14-PO), we have been investigating the utility of other markers of white matter destruction. In a collaborative study with Dr. S. Cohen (Johns Hopkins), we are examining cerebrospinal fluid for the presence of basic myelin protein. This material has been found to be elevated in the CSF of patients with a wide variety of leukoencephalopathic disorders. We are also assessing various enzymes in the CSF as possible indicators of brain tissue destruction. All patients receiving CNS prophylaxis for meningeal leukemia are serially followed with CT scans, neuro-endocrine function tests, CSF enzyme studies, and basic myelin protein determination.

Significance to Biomedical Research and the Program of the Institute

Rational treatment of central nervous system neoplasms requires knowledge of the physiology of the blood-brain barrier and a clear understanding of the CNS pharmacokinetics of antineoplastic agents. Detailed pharmacological investigation in humans is limited by the lack of a ready route of access to cerebrospinal fluid. The development of the subhuman primate model facilitates such studies in a setting that approximates the human situation. In addition, the model provides for study of chemotherapy and radiotherapy-related neurotoxicity, allowing for delineation of factors predisposing to toxicity as well as for identification of methods useful in monitoring the development of toxicity.

Data obtained in this model led to a variety of new treatment approaches now being assessed in man. Information gained in the primate regarding the levels of methotrexate achievable in the CSF following intravenous administration has led to our use of this approach in man. Data suggesting the potential advantages of intrathecal aminopterin over methotrexate have resulted in our plan to study this agent in patients. The observation that probenecid retards the disappearance of MTX from the CSF suggests that this combination may be of clinical value. Finally our finding that the hyperbaric approach to intrathecal chemotherapy yields an improved CSF drug distribution suggests that this approach should be evaluated in patients.

Proposed Course

Use of the primate model to screen agents of potential value in treating CNS malignancy will continue. Studies of a variety of agents, including the antifols, platinum compounds, nitrosoureas, radiosensitizers, and phase 1 agents (e.g., PALA) are in progress. Particular emphasis will be placed on the intravenous approach to the treatment of CNS malignancy, and studies will be designed to assess the penetration of intravenously administered compounds, e.g., methotrexate, into brain tissue. Exploration of various combination chemotherapeutic approaches to treating the CNS will also be continued. Finally, a comprehensive study of post-therapy leukoencephalopathy is underway in an attempt to learn more about those factors which predispose to the development of this syndrome.

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ANNUAL REPORT OF THE RADIATION ONCOLOGY BRANCH

NATIONAL CANCER INSTITUTE

October 1, 1977 to September 30, 1978

The past year has seen the beginning of a transition period for the Radiation Oncology Branch. After several years as the Chief of this Branch, Dr. Johnson left to go to the University of Florida and was replaced by Dr. Eli Glatstein. As a consequence of this change, many further changes are in store for the Branch.

The three main goals of the Branch are now defined explicitly as the following:

1. Major emphasis on clinical trials of combined modality predominantly collaborative with other Clinical Branches, rather than confined to within the Branch.
2. A strong radiobiology program with heavy emphasis on basic scientific and clinical questions of relevance.
3. A training program in radiation therapy capable of representing the equivalent to the outstanding programs presently going on in medical, surgical, and pediatric oncology here at the Cancer Institute.

At the present time, the thrust of investigative studies is aimed at both technological and clinical areas. The technological direction relates to the use of computerized tomography for treatment planning. This program was initially delayed in getting started because of the design of the EMI machine which initially did not permit the obtaining of a whole body contour in cross section. This has finally been corrected and the main limitation of the EMI machine at present reflects a relatively small aperture through which the patient moves. Nonetheless, with whole body contour now available, work is progressing to permit optimization of radiotherapeutic techniques. Considerable collaborative effort has gone into this project, to be detailed below as a separate item. At the clinical level, the main thrusts of present investigation reflect small cell carcinoma of the lung, along with collaborative efforts in soft tissue sarcomas with the Surgery Branch and in lymphomas with the Medicine Branch. The small cell studies have been altered in the sense that they now represent a collaborative effort with the Medicine Branch of the VA hospital. Because combined modality treatment has failed to show curative potential in patients with extensive small cell cancer, the decision has been made for patients who have disseminated disease to concentrate efforts on better chemotherapeutic regimens through the Medicine Branch at the VA. For those patients who have limited disease, it does appear as though approximately

35% of those patients can, in fact, achieve durable long-term unmaintained freedom from relapse or "cure." Our present studies with the VA are assessing the value of radiation therapy to the chest in this group of patients, since it appears as though some patients with limited disease might be cured with drugs alone.

At the present time, efforts in Hodgkin's disease and non-Hodgkin's lymphomas will be revised in conjunction with the Medicine Branch and the BCRC. Studies in chronic lymphatic leukemia have remained ongoing but our directions will be shifting shortly. Additional collaborative efforts have been made in the areas of ovarian cancer with the Medicine Branch, and pancreatic carcinoma with both Medicine and Surgery. Several pediatric tumors have been treated in collaboration with the Pediatric Oncology Branch, most notably Ewing's sarcoma, rhabdomyosarcoma, and neuroblastoma. These collaborative efforts will be described in more detail in the annual reports of the other respective Branches.

Considerable effort was made in the development of plans and design of a new radiotherapy treatment facility. This included extensive revision of initial plans and will continue with a long-range goal of renovating the present ROB space into a Radiation Biology Laboratory.

Future goals of the Branch will be focusing on collaborative efforts with Medicine, Surgery, and Pediatrics. Combined modality treatment will be the major thrust of clinical protocols; special attention will be directed in the area of radiosensitizers. This should be getting underway fairly shortly with the first tumor target being carcinoma of the esophagus. A second study will be oriented around unresectable osteogenic sarcoma and chondrosarcoma. An additional new direction of treatment is planned for intraoperative radiation therapy for advanced abdominal and thoracic neoplasms in collaboration with the Surgery Branch. This technique, which is yet to be proved, presents a unique opportunity to incorporate radiosensitizers into the treatment approach. In addition, with the tumor exposed directly to the effects of radiation at the time of surgery with critical normal tissues moved out of the field, it would also present an unusual opportunity for the application of localized heat techniques directly to the tumor.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00650-23 R0

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Service Radiation Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. H. Kent, M.D.	Head, Rad. Ther. Sec.	ROB	NCI
	K. Jentzsch, M.D.	Visiting Scientist	ROB	NCI
	M. K. Glover, R.N.	Clinical Nurse	ROB	NCI
	M. R. Powell, R.T.	Medical Radiation Tech.	ROB	NCI
	J. T. Anderson, R.T.	Medical Radiation Tech.	ROB	NCI
	A. L. Cohen, R.T.	Medical Radiation Tech.	ROB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Therapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	1.6	2.4

CHECK APPROPRIATE BOX(ES)

XX (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to provide expert radiotherapy consultation and radiation treatment for Clinical Center patients admitted to services other than the Radiation Oncology Branch fo the National Cancer Institute.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To provide consultation and radiation treatment for Clinical Center patients.

Methods Employed: Formal and informal consultation with referring physicians and application of radiation therapy where appropriate with x-rays and electrons in accordance with standard radiotherapy practice as well as modified programs where necessitated by adjuvant concomitant therapies.

Major Findings: There were 378 patients seen in formal consultation and an additional 300 logged telephone consultations provided "ad hoc" advice on treatment or general information. Approximately 424 patients will be treated in this fiscal year with over half of these being service patients, the remainder being protocol patients of the Radiation Oncology Branch.

Significance to Biomedical Research and the Program of the Institute: Provided essential radiation treatment support for ongoing clinical research programs of the National Cancer Institute and other Institutes with programs in the Clinical Center.

Proposed Course: To continue

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00684-23 RO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Non-Clinical Service Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leland R. Kirkland, Ph.D.	Research Physicist	ROB	NCI
OTHER:	Robert W. Swain	Research Physicist	ROB	NCI
	Gilbert M. Bowie		LPP	NCI

COOPERATING UNITS (if any)
Laboratory of Pathophysiology, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	.2	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Facilities and services have been provided for the irradiation of animals and biological materials required for research studies by investigators at the National Institutes of Health. Services include consultations as to methods and types of irradiation and the preparation of materials and sample holders.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To provide space, equipment and radiation physics expertise to all researchers at the National Institutes of Health who are involved with radiobiological projects.

Methods Employed: Informal consultation with all researchers who use the 250 SVp x-ray units for radiobiology experiments. Informal training of each research in the proper procedure for safely using the x-ray facility. X-ray output and energy are calibrated by project members while service for equipment is purchased from a commercial vendor.

Major Findings: Over 1000 hours of exposure time was reserved by researchers from all Institutes. Materials irradiated included cells, tissue cultures, blood parasites, mice, rats, pigs and monkeys. Preliminary results indicate that the observed biological effects in mice may be strongly influenced by which of two tubes is used to deliver the dose. Currently investigating the possibility of procuring a Cesium-157 irradiator to provide more reliable service.

Significance to Biomedical Research and the Program of the Institutes: Provides essential radiation physics support for ongoing research of the National Cancer Institute and other Institutes.

Proposed Course: To be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00988-13 RO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Study of Chronic Lymphocytic Leukemia (CLL)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. D. Lamberg, M.D.	Senior Investigator	ROB	NCI
OTHER:	Staff of the Rad. Oncol. Branch		ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The initial purpose of this project was to study the natural history of chronic lymphocytic leukemia (CLL), to define clinically important disease parameters and to investigate new therapeutic approaches. Total body irradiation (TBI) alone or with chemotherapy has been shown to yield Type-I response in greater than one-third of patients, and this type of response has been correlated with prolonged survival. Commonly employed regimens using alkylating agents and steroids give significantly lower Type I response rates and survival rate. All studies to date have employed historical controls, and have not stratified treatment response with regard to stage of disease. This study has been redirected to examine the therapeutic benefit of TBI and chemotherapy vs. chemotherapy alone in a prospective, randomized, staged group of patients with advanced CLL in whom therapeutic intervention is justified.

Project Description:

Objectives: By comparing the TBI pilot study yielding the best Type I response rate in CLL (a regimen consisting of TBI plus chemotherapy) with the community standard of chemotherapy alone, this study will (1) test the efficacy of regimens employing TBI with those not employing TBI and will (2) examine the role of TBI in the general management of CLL.

Methods Employed: Patients with untreated CLL of any stage will be accessioned to the study and will be followed until therapy is necessitated by the appearance of Stage III or IV CLL, at which time the patients will be randomized and treated. They will also be monitored immunologically prior to and during therapy.

Major Findings: Pilot studies employing TBI have shown that it may be the most effective agent to date in the management of CLL.

Significance to Biomedical Research and the Program of the Institute: Because peripheral blood contains the tumor tissue, giving easy access to cells; because of its protracted course, and because of the immunologic aberrations associated with CLL; this disease offers the opportunity to demonstrate efficacy of therapeutic measures and at the same time the opportunity to investigate the role of immunologic parameters in determining the responsiveness. A number of assays have recently been developed in our lab which appear to be useful in characterizing CLL patients, and which may prove to be effective monitors or prognosticators of responsiveness.

Proposed Course: The study will begin to accession patients to the randomized prospective study as soon as the new protocol is approved.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00990-13 RO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Study of Non-Hodgkin's Lymphomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eli Glatstein, M.D.	Acting Chief	ROB	NCI
OTHER:	H. D. Brereton, M.D.	Head, Rad. Med. Sec.	ROB	NCI
	Staff of the Rad. Oncol. Branch		ROB	NCI

COOPERATING UNITS (if any)
Medicine Branch, NCI
Laboratory of Pathology, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to increase our understanding of the natural history of the various malignant lymphomas, to define which diagnostic (staging) methods are requisite for making treatment decisions, and to evaluate various therapeutic approaches which might improve the survival for patients with these diseases.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To improve our understanding of the natural history of malignant lymphomas, to define which staging methods are required for effective treatment decision-making, and to evaluate new therapeutic approaches with the aim of improving survival and freedom from relapse.

Methods Employed: Extensive prospective staging has been completed on well over two hundred patients, which patients have subsequently been accessioned into clinical trials to examine the comparative value of different therapeutic approaches.

Major Findings: The systemic nature of involvement in the majority of patients with non-Hodgkin's lymphoma has been established by this collaborative study with the Medicine Branch and Laboratory of Pathology. This information has served to alter the concepts regarding treatment and the more routine use of systemic therapy has resulted. However, while remissions can be achieved in the majority of patients using either radiotherapy or chemotherapy, relapse eventually occurs in most patients and the investigation of alternative therapeutic measures will be required. The current study demonstrates that a combination of total body irradiation with combination chemotherapy (CVP or C-MOPP) is no more efficacious than combination chemotherapy or total body irradiation alone in achieving remissions and long-term survival.

Significance to Biomedical Research and the Program of the Institute: This study has contributed to our understanding of the non-Hodgkin's lymphoma primarily in terms of understanding the natural history and providing information as to methods of staging patients (i.e., identifying their location in the overall spectrum of disease). The remaining challenge is to develop more effective means of therapy in the future.

Proposed Course: Continued accession of patients is anticipated for the purpose of examining new treatment approaches, concentrating on (1) when to intervene therapeutically in patients with advanced nodular lymphoma and (2) what role for radiation to nodes exists in patients in whom a complete remission has been induced by chemotherapy.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00994-07 RO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
The Study of Small Cell Cancer of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. D. Brereton, M.D.	Head, Rad. Med. Sec.	ROB	NCI
OTHER:	C. H. Kent, M.D.	Head, Rad. Ther. Sec.	ROB	NCI

COOPERATING UNITS (if any)
Diagnostic Radiology Dept., CC, NIH
Nuclear Medicine Dept., CC, NIH

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Medicine Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	.5

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
This study is concerned with the evaluation of methods for pretreatment assessment of patients, identification of factors influencing response to therapy, and investigation of combined modality treatment using radiotherapy and intensive chemotherapy.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The major objectives of the program is to improve the survival time for patients with small cell carcinoma of the lung and hopefully to develop treatment programs which may offer some prospect of cure.

Methods Employed: Eighty-six patients with previously untreated disease have been treated between November 1974 and December 1977. A diagnostic workup consisting of physical examination, whole lung tomography, bone marrow biopsy, and radioisotope scans of bone, liver, and brain has been employed to divide the cases into two categories, i.e., those with disease limited to the chest (intrathoracic group) and those with extrathoracic metastases. The therapeutic approach utilizes combination chemotherapy, whole brain irradiation, and radiotherapy to regional disease.

Major Findings: The complete remission rate for intra- and extrathoracic disease has been approximately 80% and 50% respectively. Following the induction course of treatment, lasting 3-4 months, patients have been observed without further treatment when a complete remission was noted. The duration of remission for patients with extrathoracic disease has been relatively short without maintenance therapy (median 7.5 months) but survival has been significantly prolonged (median 16.5 months) as compared to historical controls. In contrast, durable unmaintained remissions have been observed in patients with intrathoracic disease who achieved complete remissions and the median survival has been dramatically prolonged (median in excess of 3 years).

Significance to Biomedical Research and the Program of the Institute: The durable remissions in patients with more limited disease suggests the possibility of cure in this heretofore fatal form of lung cancer.

Proposed Course: A cooperative study between the NCI-VA Group and the Radiation Oncology Branch was initiated in January, 1978. All patients are referred to the VA Hospital for initial work-up and staging. Half of the patients with intrathoracic disease at presentation will receive radiation therapy to the primary only plus concomitant chemotherapy in order to more accurately define the role of radiation therapy in the management of patients with oat cell carcinoma of the lung as opposed to chemotherapy alone. To date, 6 patients have been randomized to this combined modality study. Preliminary results appear to be encouraging but it is too early to evaluate the results at this time.

Publications:

Brereton, H.D., O'Donnell, J.F., Kent, C.H., Matthews, M., Dunnick, N.R., Johnson, R.E.: Spinal meningeal carcinomatosis in small cell carcinoma of the lung. Ann.Int. Med. (In Press).

Brereton, H.D., Matthew, M., Costa, J., Kent, C.H., Johnson, R.E.: Mixed anaplastic small cell and squamous cell carcinoma of the lung. Ann.Int.Med. (In Press).

Brereton, S.A., Kent, C.H., Costa, J., Webber, B., Young, R.E., Chabner, B., Seipp, C., Simon, R.: Prospective randomized evaluation of the role of limb sparing surgeon, radiotherapy and adjuvant chemoimmunotherapy in the treatment of adult soft tissue sarcomas. Surgery. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00995-03 RO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Radiation Therapy Planning Using Computerized Tomography Interfaced with Computerized Dosimetry Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. R. Kirkland, Ph.D.	Physicist, Head	ROB	NCI
OTHER:	Eli Glatstein, M.D.	Chief	ROB	NCI
	D. Syed, M.S.E.E.	Head, CSL	CR	NIH
	W. L. Risso, M.E.	Elec. Engineer	CR	NIH
	H. A. Fredrickson	Computer Systems Analyst	CR	NIH
	J. DeLeo	Computer Systems Analyst	CR	NIH

COOPERATING UNITS (if any)
Computer Systems Laboratory, CR, NIH

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	3.6	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is directed toward utilizing computerized tomography and ultrasound to improve radiation therapy treatment planning. A whole-body x-ray scanner and a PDP-11/70 computer have been installed. Multiple CT images can be simultaneously displayed on one video monitor as a function of either image spatial sequence or time. Automatic contouring with quick edit option has been implemented. Contour area, mean density, and density histogram can be automatically produced on monitor. Distances between points can be calculated in real dimensions to provide accurate definition of tumor depths.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To utilize the imaging from computerized tomography scans and ultrasound scans as part of service medical physics for improved radiation treatment planning and to employ data on tissue densities to correct for tissue inhomogeneities.

Methods Employed: A closely integrated effort is being undertaken by the staff of the Radiation Oncology Branch and the Computer Systems Laboratory. The whole body scanner and treatment planning computer are functional and the ultrasound interface is being connected. Treatment planning software has been modified to require a minimum of keyboard input by the operator and beam data for existing therapy devices have been stored in memory. Efficacy of treatment planning is being compared for patients for the cases when no CT scan is available, when only a diagnostic CT scan is available and when a CT scan adapted for radiotherapy is available.

Major Findings: Computer-to-computer link would simplify image transfer from scanner to treatment planning system. Introduction of 16" wedges on scanner permits automatic contouring of virtually all patients. Selection of planning plane is simplified by multiple scan display. Temporal progress of treatment can be monitored by using same multiple scan display in conjunction with software that computes contour area and density. Video image distortion of distances can be circumvented by measuring distances in "CT number" space and converting to real space. Existing hardware is compatible with an experimental digital ultrasound system located outside of Branch. This compatibility will facilitate the comparison of the efficacy of x-ray CT scans versus ultrasound scans for radiotherapy treatment planning.

Significance to Biomedical Research and the Program of the Institute: A successful outcome of this program will improve the accuracy of treatment planning, hence dose delivery, to patients undergoing therapy on research protocols.

Proposed Course: Expedient prosecution.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00996-02 RO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Immunologic Function in Patients Receiving Total Body Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. D. Lamberg, M.D.	Senior Investigator	ROB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Medicine Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	.75	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Total Body Irradiation has been shown to cause striking regression of disease in chronic lymphocytic leukemia (CLL) and in non-Hodgkin's lymphoma (NHL). The time course of the response, particulary in NHL, suggests that mechanisms other than radiation-induced cell death may be involved, and further that the mechanisms may be immunologic. The time course of response, 6-8 weeks in NHL and 6-12 months in CLL, are such that these diseases also become suitable models in which to develop and test serial immunologic monitoring techniques. This study was initiated in order to: (1) develop tests of immunologic function which are sufficiently reproducible, and sensitive to detect alterations in immune function induced by therapy and/or change in disease status; and (2) apply these tests in a situation where it is likely that one may be able to assess the relative contributions of therapy and level of immune function to change in disease status.

Project Description:

Objectives: Although many assays of immunologic function have been developed and applied in the last decade, inattention to many relevant biological and technical factors has made the interpretation of their significance and relevance almost impossible. This situation is further complicated by the tendency of many studies to assume at the outset that immunologic alterations are the relevant alteration in the outcome of the disease process, and then to present data on immunologic alterations as the proof of this pre-drawn conclusion of relevance.

Although a body of knowledge has been developed which suggests that immune function and the development of cancer are related, the exact nature of this relationship, and ways in which it may be exploited to clinical advantage are largely unknown. If one is to approach the question scientifically, one first needs to develop reliable, reproducible tests which can serve as true yardsticks for testing of hypotheses.

Total Body Irradiation has been shown to cause striking regression of disease in chronic lymphocytic leukemia (CLL) and in non-Hodgkin's lymphomas (NHL). The time course of response, particulary in NHL, suggests that mechanisms other than radiation induced cell death may possibly be involved and further that the mechanisms might have an immunologic basis. Studies have shown that Total Body Irradiation causes immunological depression and recovery in rats, (Lamberg, 1971) and its time course is similar to that of response in NHL. The time course of response, 6-8 weeks in NHL and 6-12 months in CLL are such that these diseases also become suitable models in which to develop and test serial immunologic monitoring techniques.

This study was initiated in order to (1) develop tests of immunologic function which are sufficiently reproducible and sensitive to detect alterations in immune function induced by therapy and/or change in disease status and (2) apply these tests in a situation where it is likely that one may be able to assess the relative contributions of therapy and level of immune function to change in disease status.

Methods Employed:

(1) Skin testing
(2) *In vitro* blastogenesis
(3) T & B cell analysis using rosetting
and immunofluorescence techniques

Major Findings: Initial studies indicate that (1) serum factors, which have usually been held unimportant, have a significant impact on the in vitro reactivity to blastogenic assays as measured with tritiated thymidine uptake as an endpoint; (2) tritiated thymidine uptake is a poor endpoint for blastogenic assays; (3) automated methods of counting cell number may be used as an endpoint for blastogenic assays and also for quantitating rosette formation by T-lymphocytes with sheep red blood cells (SRBC's); (4) cells from peripheral blood of patients with CLL and also lymph node cells of patients with NHL form rosettes when mixed with SRBC'S which have been presensitized with calf serum. The sensitizing factor appears to be antibody and it is presently being characterized. Whereas the rosetting fraction in the CLL and NHL have been 35%-91%, the highest fraction observed in normal peripheral blood has been 25%. This may prove to be a useful marker for B cell neoplasms and in addition, since it too has been automated, may prove to be a useful means of monitoring the course of these diseases.

Significance to Biomedical Research and the Program of the Institute: A considerable developmental research effort has been based on the assumption of the relevance of immune function to carcinogenesis and cancer therapy. Nevertheless, the initial fruits of such research, as presented at the recent Immunotherapy Symposium (Terry, 1976) were indeed disappointing. It is time for critical thinking and for the development of objective and sensitive yardsticks for hypothesis testing. The particle counting system developed in our laboratory is likely to provide tests meeting this need. To proceed rationally we need accurate and reproducible tests by which to analyse immune function. We may then be in a position to analyse mechanisms of failure in immunotherapy trials, and also to do the same in trials employing immune modulation.

Proposed Course: The methods which have been recently developed will be employed in normal subjects and patients and then subjected to technical analysis when approximately 10 patients and normals have been studied. Thereafter, tests will be performed until statistically significant correlations between treatment, immune status and disease response have been established or refuted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00997-02 RO

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Service Radiation Physics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. R. Kirkland, Ph.D.	Head, Rad. Physics Section	ROB	NCI
OTHER:	Robert Swain	Research Physicist	ROB	NCI
	R. Whittaker	Engineering Technician	ROB	NCI
	M. R. Powell, R.T.	Medical Radiation Technician	ROB	NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2.0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Services and equipment have been provided for expert consultation in radiation physics, radiation therapy treatment planning and radiation treatment for Clinical Center patients admitted to services other than the Radiation Oncology Branch of the National Cancer Institute.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To provide equipment and consultation for the radiation therapy of Clinical Center patients.

Methods Employed: Linear accelerators were calibrated and maintained to provide accuracy in the radiation dosimetry for patients undergoing radiation therapy. Computers were employed to generate actuarial survival curves and treatment plans in which x-ray beam configurations are varied to determine the optimum for patient care. Informal consultations were held with radiotherapists concerning energy, modality and configurations of beams.

Major Findings: A new form was produced for the recording of patient treatments and reaction which facilitates the daily monitoring of dose levels. The whole skin treatment of patients was introduced following the development of an unique rotating platform which gives an improved dose distribution over the entire skin. The method for treating Hodgkin's disease was drastically altered to permit beam blocking close to the patient and to adopt methods used routinely at other therapy centers. A radiotherapy simulation was purchased, installed and commissioned to provide the rapid and accurate localization of treatment beam ports. Equipment specifications were developed for the selection of 4 new accelerators to be used to treat patients.

Significance to Biomedical Research and the Program of the Institute: Provides essential radiationphysics support for ongoing clinical research programs of the National Cancer Institute and other Institutes with programs in the Clinical Center.

Proposed Course: To continue.

Publications: None

ANNUAL REPORT SUMMARY

SURGERY BRANCH

NATIONAL CANCER INSTITUTE

October 1, 1977 to September 30, 1978

Clinical efforts in the Surgery Branch continue to emphasize combined modality approaches to the treatment of cancer. Active clinical protocols are in progress for the treatment of osteosarcoma, soft tissue sarcomas, melanoma, rectal cancer, testicular cancer, and lung cancer. Immunotherapeutic approaches to cancer treatment are being explored. Active laboratory research programs in tumor immunology and tumor metabolism are in progress.

Surgical procedures and consultations performed by the Surgery Branch from April 1, 1977 to March 31, 1978 are presented in Tables I to IV.

TABLE I
Cases - General Surgery*
April 1, 1977 - March 31, 1978

General:	Staging laparotomy and splenectomy for lymphoma	6
	Aortocaval lymph node dissection and biopsy for ovarian cancer staging	16
	Exploratory laparotomy	23
	Splenectomy	5
	Colon/bowel resection	22
	Abdominal-perineal resection	4
	Total pelvic exenteration	2
	Appendectomy	2
	Gastrectomy	1
	Pancreatectomy - partial	4
	Excision pilonidal cyst	2
	Cholecystectomy	17
	Major soft tissue or muscle group excision	13
	Revision of colostomy	1
	Creation loop colostomy	1
	Herniorrhaphy/hernia repair	10
	Wound exploration	4
	Electrofulguration - rectum	8
	Colonoscopy	19
	Laparoscopy	7
	Peritoneoscopy	3
Surgery for Melanoma:	Wide excision with/without STSG	20
	Wide excision + axillary LND	19
	+ inguinal LND	11
	+ radical neck LND	8
	Lymph node dissection: Axillary	14
	Superficial inguinal	6
	Deep inguinal	6
	Radical neck	7
Head & Neck:	Radical neck ± other resection	3
	Other major cases	4
	Tracheostomy	7
	Direct laryngoscopy/pharyngoscopy	2
Plastic:	Wound debridement - major	16
	Construction/revision/movement - flaps	5
	STSG	3
	Electrofulguration skin lesions	2

TABLE I (continued)

GU:	Aortocaval lymph node dissection	14
	Debulking procedure	5
	Orchiectomy/orchiopexy	7
	Nephrostomy/nephrolithotomy/nephrectomy	10
	Transureteroureterostomy/ureterolithotomy	5
	Pyelolithotomy	2
	Suprapublic prostatectomy	2
	TURP	7
	Repair varicocele	2
	Hydrocelectomy	2
	Loop revision	2
	Phalloplasty	2
	Circumcision	2
	Marshall-Marchetti	1
	Looposcopy	2
	Cystoscopy with/without other studies, or biopsy	55
	Cystometrogram	20
	Ureterogram	4
	Testicular biopsy	12
	Miscellaneous GU biopsies	3
Breast:	Breast biopsy	54
	Simple mastectomy	3
	Modified radical mastectomy	13
	Reduction mammoplasty	1
GYN:	Hysterectomy	3
	Salpingo-oophorectomy	4
	D and C	3
	Tubal ligation	2
Amputations:	Below knee	4
	Above knee	8
	Hip disarticulation	2
	Hemipelvectomy	10
	Shoulder disarticulation	1
	Forequarter amputation	2
	Stump revision	2
	Forearm	1
	Ray	1
Endocrine:	Parathyroidectomy	22
	Parathyroid implant or removal	7
	Adrenalectomy	14
	Thyroidectomy - complete or subtotal	17
	Radical neck LND - met. thyroid ca.	4

TABLE I (continued)

Vascular:	Venous bypass	3
	Endarterectomy	4
	A-V shunt/shunt revisions/shunt removal	27
Thoracic:	Unilateral thoracotomy:	
	Biopsy nodule(s)	53
	Biopsy nodule(s) with rib resection/chest wall	6
	Lobectomy	7
	Bilateral thoracotomy	2
	Pericardiectomy	2
	Transthoracic hernia repair	2
	Resection mediastinal mass	3
	Bronchoscopy	12
	Mediastinoscopy	3
Minor:	Incision and drainage of abscess	12
	Biopsy: Temporal artery	1
	Bone	20
	Node	91
	Tissue mass	68
	Insertion abdominal catheter for chemotherapy	4
Miscellaneous:		21

*In compiling this list the major aspect of each procedure was used. Patients with multiple simultaneous procedures appear on the list only one time.

TABLE II
Consultants - Surgery
April 1, 1977 - March 31, 1978

Plastic:	
Mammoplasty	6
Skin grafting, extensive	6
Revision/excision scar	5
Thoracic:	
Pericardiectomy	2
Bronchoscopy	2
Thoracotomy - nodules	3
Thoracotomy - infiltrates	5
Lobectomy	1
Mediastinoscopy	1
Rib resection	1
Miscellaneous	2
GYN:	
Exploratory laparotomy	3
Cervical biopsy	2
Hysterectomy	2
D and C	9
Salpingectomy/oophorectomy	2
Miscellaneous	3
ENT:	16
Neurosurgical:	2
Orthopedic:	5

TABLE III

Individual Written Intramural Consultations Received by Surgery Branch, NCI Listed by Institutes*

April 1, 1977 - March 31, 1978

NCI	262
NIAMDD	105
NHLBI	65
NIAID	68
NIMH	35
NINCDS	49
NIDR	13
NICHHD	17
NEI	6
TOTAL	620

*This tabulation includes only formal written consultations submitted to the Surgery Branch office. Many additional consultations are received by Surgery Branch physicians directly by telephone or personal contact. These are not included in this tabulation.

TABLE IV

Annual Statistical Report

Surgical Nursing Service

April 1, 1977 - March 31, 1978

Patient		NCI	NHLBI	NINCDS		NIAID	NIAMDD	NEI	NIMH	NICHHD	NIDR	Consult-	Total	#Pts.
				Neuro.	Muscle							ants	Operated	
NCI	Major	672	3	15	–	–	7	–	–	–	–	20[a]	717	918
	Minor	194	–	–	2	–	2	–	–	–	–	3	201	
NHLBI	Major	34	289	–	–	–	–	–	–	2	–	5[b]	330	362
	Minor	18	13	–	1	–	–	–	–	–	–	–	32	
NINCDS	Major	16	19	39	29	–	–	1	–	2	–	–	106	348
	Minor	9	–	2	231	–	–	–	–	–	–	–	242	
NIAMDD	Major	86	1	2	1	–	16	–	–	1	–	12[c]	119	163
	Minor	22	–	–	9	–	12	–	–	–	–	1	44	
NIAID	Major	31	3	3	–	–	–	4	–	1	–	2	44	67
	Minor	13	–	–	5	–	–	1	–	–	–	4	23	
NIDR	Major	1	–	–	–	–	–	–	–	–	4	–	5	6
	Minor	–	–	–	1	–	–	–	–	–	–	–	1	
NIMH	Major	19	–	–	–	–	1	–	–	–	–	–	20	22
	Minor	2	–	–	–	–	–	–	–	–	–	–	2	
NICHHD	Major	14	–	1	–	–	–	–	–	3	–	4	22	24
	Minor	2	–	–	–	–	–	–	–	–	–	–	2	
NEI	Major	2	–	–	–	–	–	12	–	–	–	–	14	24
	Minor	–	–	–	4	–	–	16	–	–	–	–	10	
MAJOR TOTAL		875	315	60	30	0	24	17	0	9	4	43	1377	
MINOR TOTAL		260	13	2	253	0	14	7	0	0	0	8	557	
GRAND TOTAL		1135	328	62	283	0	38	24	0	9	4	51	1934*	

*Of the 1934 surgical procedures, 178 were on O.P.D. status; 9 were performed on 6W Nursing Unit; 24 were performed by the Anesthesiology Department.

[a] Incl. 6 Anes.

[b] Incl. 4 Anes.

[c] Incl. 10 Anes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03800-08 SURG

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Surgical Consultations and Collaborative Research Involving Surgical Services at the National Institutes of Health

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Rosenberg	Chief of Surgery, NCI	SURG	NCI
	and entire staff of	Surgery Branch, NCI	SURG	NCI
Other:	G. D. Aurbach	Chief, Metabolic Dis. Br.	MD	NIAMDD
	F. C. Bartter	Clinical Director	IR OD	NHLBI
	J. L. Doppman	Chief, Diag. Radiol. Dept.	DR	CC
	R. E. Johnson	Chief, Rad. Oncol. Br.	RO	NCI
	J. Robbins	Chief, Clin. Endocrin. Br.	CE	NIAMDD
	L. B. Thomas	Chief, Lab. of Pathology	LP	NCI
	R. C. Young	Chief, Medicine Branch	M	NCI
	A. S. Levine	Chief, Ped. Oncol. Br.	PO	NCI
	J. Gardner	Chief, Digestive Dis. Br.	DD	NIAMDD

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients for elective consultations as well as all emergency general surgical problems. Many collaborations on clinical studies have resulted from these consultative efforts.

PHS-6040
(Rev. 10-76)

Introduction

Investigators in the Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients in primarily two capacities. Firstly, we see patients in consultation for all general surgical and specialty surgical problems except for the specialties of cardiac and orthopedic surgery. The Surgery Branch answers all emergency as well as elective surgical consultations and provides 24 hour coverage for surgical emergencies that may arise in the Clinical Center Hospital.

Secondly, the Surgery Branch collaborates in the procurement of tissues for studies required by other investigative units. The degree of involvement of the Surgery Branch in the planning and execution of these studies is variable. The Surgery Branch often plays an instrumental role in the design of these studies while in other collaborations, the Surgical Service merely provides tissues.

Approximately 40% of the clinical surgical effort of the Surgery Branch is devoted to these consultative and collaborative studies.

A complete listing of surgical procedures performed by the Surgery Branch is presented in Table I. Surgery performed by surgical consultants operating within the Surgery Branch is listed in Table II.

A list of consultations received from other NCI Branches as well as other NIH Institutes is shown in Table III.

Project Description: Selected projects are presented below to provide examples of the nature of Surgery Branch collaborative efforts.

Part I.	Hyperparathyroidism
Part II.	Reduction Mammoplasty
Part III.	Nutritional Support
Part IV.	Ovarian Cancer
Part V.	Tenckhoff Catheters

Part I. Hyperparathyroidism

In the period 4/15/77 to 4/15/78, 24 patients were operated on for hyperparathyroidism. Twelve of the 24 had previously been operated on unsuccessfully prior to referral. One of those patients fails to be relieved of his hypercalcemia. Considerable progress has also been made in parathyroid autotransplantation and *in vitro* assessment of parathyroid tissue function.

Part II. Reduction Mammoplasty

This program, in conjunction with the National Institute of Child Health and Human Development, is intended to afford cosmetic mammary reduction for men under study for causes of gynecomastia. Frequently these reductions can be done through a circumareolar incision with minimal scarring. Occasionally transposition of the nipple-areolar complex and excision of skin is necessary to obtain a satisfactory appearance. Adequate specimens of breast tissue and skin are made available for hormone receptor analysis, culture, and biomedical studies.

Part III. Nutritional Support

The Surgery Branch continues to mount a major effort in supporting patients nutritionally by intravenous feeding throughout the National Institutes of Health. The Branch has been responsible for 984 days of total parenteral nutrition between the periods of 4/15/77 and 4/15/78.

Part IV. Ovarian Cancer

Studies of ovarian carcinoma are undertaken in Medicine Branch protocols, with the cooperation of the Surgery Branch. Adjuvant systemic melphalan chemotherapy is being compared with intraperitoneal radioactive phosphorus for high-risk patients following complete surgical tumor resections. Patients with incomplete resections are surgically debulked and treated with systemic chemotherapy alone or with a combination of chemotherapy and pelvic irradiation. The Surgery Branch collaborates with the Medicine Branch in ovarian cancer studies by providing surgical evaluations and services, as well as performing definitive surgical explorations and resections as required in protocols. In 1977, the Surgery Branch performed operative procedures on 16 patients in ovarian cancer protocols.

Part V. Tenckhoff Catheters

The Surgery Branch has been responsible for the placement of a number of Tenckhoff catheters for intraperitoneal dialysis with chemotherapeutic agents. The primary thrust in this has been the use of methotrexate in ovarian cancer, but it has recently been extended to use in control of prolonged ascites and for administration of other agents such as 5-FU.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03801-08 SURG

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Clinical Studies in Cancer Surgery

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Rosenberg	Chief of Surgery, NCI	SURG	NCI
	and entire staff of	Surgery Branch, NCI	SURG	NCI
Other:	L. B. Thomas	Chief, Lab. of Pathology	LP	NCI
	W. Terry	Chief, Immunology Br.	I	NCI
	R. C. Young	Chief, Medicine Branch	M	NCI
	B. A. Chabner	Head, Biochem. Pharm. Sect.	LCHPH	NCI
	M. Lippman	Senior Investigator	M	NCI
	R. E. Johnson	Chief, Rad. Oncol. Br.	RO	NCI
	J. L. Doppman	Chief, Diag. Radiol. Dept.	DR	CC

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 10.6	PROFESSIONAL: 7.6	OTHER: 3.0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgery Branch has a variety of studies investigating innovative therapies for patients with malignant disease. The major emphasis of these studies is in the treatment of malignant melanoma, soft tissue sarcomas, osteogenic sarcomas, and rectal cancer. The major emphasis in Surgery Branch cancer therapy is in adjunctive therapy with emphasis on the use of multiple treatment modalities in addition to surgery.

PHS-6040
(Rev. 10-76)

Project Description:

Part I.	Malignant Melanoma
Part II.	Soft Tissue Sarcomas
Part III.	Osteogenic Sarcoma
Part IV.	Testicular Cancer
Part V.	Colorectal Cancer
Part VI.	Total Parenteral Nutrition
Part VII.	Thymosin
Part VIII.	Quality of Life Studies
Part IX.	Endoscopy
Part X.	Computer Applications

Part I. Malignant Melanoma

Surgery Branch studies in malignant melanoma include studies of primary melanoma by the intralesional injection of BCG and the treatment of recurrent cutaneous melanoma by the intralesional injection of DNCB. A prospective randomized study is evaluating the efficacy of deep lymph node dissection in patients with lower extremity melanoma. In conjunction with the Immunology and Medical Oncology Branches we are conducting a prospective randomized study of three adjuvant treatments in addition to surgery for the treatment of patients with Stage II melanoma.

Part II. Soft Tissue Sarcomas

The Surgery Branch is conducting a prospective randomized study of the efficacy of radical surgery and adjuvant chemotherapy in the treatment of patients with soft tissue sarcomas. Fifty-five patients have been entered into this study. Significant improvement in disease-free survival appears to result from the use of adjuvant chemotherapy.

Part III. Osteogenic Sarcoma

The Surgery Branch is prospectively evaluating the use of high-dose methotrexate for the adjuvant treatment of patients with osteosarcoma. This adjuvant chemotherapy appears to significantly improve disease-free survival. Aggressive thoracotomy for the treatment of pulmonary nodules appears to significantly improve overall survival.

Part IV. Testicular Cancer

This protocol is designed to determine whether surgical resection of all accessible tumor (debulking) combined with chemotherapy can prolong the response rate and increase the survival rate of selected patients with advanced metastatic germ cell tumors of the testis, compared to chemotherapy alone. Only patients with embryonal carcinoma or teratocarcinoma with tumor bulk accessible to surgical resection are eligible. So far 19 patients have been entered into the prospective randomized protocol. The data are too preliminary to draw any conclusions concerning the effectiveness of debulking surgery in improving the survival of these patients.

Part V. Colorectal Cancer

These studies are designed to evaluate in a randomized, prospective fashion the role of electrofulguration alone and in combination with immunotherapy in the definitive treatment of rectal cancer and to assess the sensitivity and accuracy of preoperative laparoscopy in determining those patients who present with Stage D (disseminated) rectal cancer. In May 1976, the Surgery Branch activated a three-armed study designed to compare electrofulguration alone or electrofulguration plus adjuvant BCG immunotherapy with abdominoperineal resection. To date 29 patients have been evaluated for this study. Eight have been seen in the past year. There are seven patients currently on study; APR (3), electrofulguration alone (3), and electrofulguration plus BCG (1).

Part VI. Total Parenteral Nutrition

As an adjunct to aggressive chemotherapy the Surgery Branch is involved in two prospective randomized studies to examine the efficacy of parenteral nutrition in conjunction with aggressive chemotherapy. These are a pediatric adolescent group of metastatic soft tissue and bony sarcomas undergoing ablative chemotherapy and a prospective study using TPN as a supportive adjunct to a very aggressive induction and consolidation phase for diffuse histiocytic lymphoma.

Part VII. Thymosin

Previous studies conducted in the Surgery Branch suggested that cancer patients with low levels of cellular immunity may benefit clinically from thymosin during conventional tumor treatment. To test this hypothesis a randomized trial in patients with small cell carcinoma of the lung who received intensive immunosuppressive induction chemotherapy during the initial six weeks of treatment was conducted by the VA Medical Oncology Branch with the collaboration of the Surgery Branch. The patients were randomized to receive thymosin 60 mg/m^2, thymosin 20 mg/m^2, or a placebo twice a week during induction chemotherapy. Fifty-five patients were entered on the protocol ending January 4, 1977. There was no statistically significant difference among groups in clinical response to treatment. However, survival of the T_{60} group was significantly longer than that of the P group ($p<.01$).

The median survival (in days) was 424, 255, and 243 for the T_{60}, T_{20}, and P groups, respectively. The significantly increased survival of the T_{60} patients was due to a prolongation of the complete response in these patients. Thus, in this trial, thymosin did not influence clinical response to chemotherapy but was associated with a prolongation of the interval of the complete response in patients receiving a complete response to chemotherapy.

Part VIII. Quality of Life Studies

Although survival statistics in the various arms of a controlled clinical trial are not difficult to obtain, the change that various treatment regimens cause in the quality of life is more difficult to quantitate. Therefore, a Quality of Life Assessment (QOLA) has been constructed to measure the economic, sociologic, and psychologic cost of cancer treatments. This cost of therapy is considered to be valuable data useful to the physician who recommends a particular treatment plan to a patient. At present a retrospective study of patients undergoing therapy for soft tissue sarcoma is underway; a comparison of the quality of life change in patients undergoing a major amputation to those receiving limb-sparing surgery plus high-dose radiation therapy is being made. All patients studied to date received chemotherapy. A prospective study of the impact of cancer treatment on quality of life is being planned.

Part IX. Endoscopy

The Surgery Branch continues to collect clinical data to help define the appropriate role for laparoscopy in cancer diagnosis and treatment. Laparoscopic tubal ligation, as a consultative service, is available for appropriate patients. During the past year, 30 patients have undergone laparoscopy on the NCI surgical service. Activation of the Surgery Branch colon and rectal cancer protocols promises to increase our utilization of this endoscopic procedure in the coming year.

Fiberoptic endoscopy of the lower GI tract is available as a consultative service function of the Surgery Branch. The utility of this procedure from both the diagnostic and therapeutic point of view of colon lesions is well established. Twenty-one colonoscopic procedures were performed in the past year. These included 11 diagnostic examinations and 10 snare polypectomies. Direct visual access to and ability to histologically sample tissues from the entire lower GI tract offer excellent opportunities for earlier diagnosis of colon malignancy. Premalignant lesions in patients with ulcerative colitis can be identified. Colonoscopy will play a role in the preoperative assessment of appropriate patients to undergo colon and rectal surgery.

Part X. Computer Applications

Data for Surgery Branch research continued to be collected and entered into three primary systems: (a) randomized protocols, (b) serum/cell inventory, and (c) nutrition studies. The basic objectives underlying the design of these systems are to: (1) ensure very high accuracy of the data, and

(2) permit information to be easily recorded, keyentered, verified, corrected, retrieved, and analyzed.

These systems are maintained on computers at the Division of Computer Resources and Technology. On the IBM-370 system, the text-editor WYLBUR is used for data entry, program maintenance, and remote job submission. The Time Sharing Option is used for graphic displays. On the DECsystem-10 computer, various software packages are used for entry of laboratory data and also for graphic displays. A Tektronix 4012 terminal was acquired by the Branch during the last year to help fulfill the increasing demand for data retrieval and graphic display of information. An IBM-2741 terminal continues to be used for data entry and job submission.

A simple measure of data processing activity is to report the current record counts for the various systems. The following counts show the status of the three systems as of June 1978:

	System	No. of Protocols or Studies	No. of Subjects	No. of Records
(a)	Randomized protocols	9	285	6,217
(b)	Serum/cell inventory	24	5,795	44,590
(c)	Nutrition studies	6	189	20,064

In the serum/cell inventory there are currently 35,058 vials of serum and 7,048 vials of lymphocytes collected in 13,077 drawings.

Data processing will continue to play a significant role in assisting Surgery Branch investigators to define and describe the characteristics of protocol populations.

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Au, F.C., Webber, B., and Rosenberg, S.A.: Pulmonary granulomas induced by BCG. Cancer (in press).

Bergman, S.M., Sears, H.F., and Javadpour, N.: A complication of using the mechanical stapling device in the creation of an ileoconduit. Urology (in press).

Chang, A.E., Schaner, E.G., Conkle, D.M., Flye, M.W., Doppman, J.L., and Rosenberg, S.A.: Evaluation of computed tomography in the detection of pulmonary metastases: A prospective study. Cancer (in press).

Rosenberg, S.A., Kent, H., Costa, J., Webber, B.L., Young, R., Chabner, B., Baker, A.R., Brennan, M.F., Chretien, P.B., Cohen, M.H., deMoss, E.V., Sears, H.F., Seipp, C., and Simon, R.: Prospective randomized evaluation of the role of limb sparing surgery, radiation therapy and adjuvant chemotherapy in the treatment of adult soft-tissue sarcomas. Surgery (in press).

Rosenberg, S.A., Seipp, C., and Sears, H.F.: Clinical and immunologic studies of disseminated BCG infection. Cancer (in press).

Sindelar, W.F., Bagley, D.H., Felix, E.L., Doppman, J.L., and Ketcham, A.S.: Efficacy of full-lung tomography in screening cancer patients for pulmonary metastases. JAMA (in press).

Sindelar, W.F., Costa, J., and Ketcham, A.S.: Osteosarcoma associated with thorotrast administration: Report of two cases and literature review. Cancer (in press).

Weingrad, D.N., and Rosenberg, S.A.: Early lymphatic spread of osteogenic and soft tissue sarcomas. Surgery (in press).

Cohen, M.H., Jessup, J.M., Felix, E.L., Hume, R.B., Weese, J.L., Herberman, R.B., and Rosenberg, S.A.: Intralesional treatment of recurrent metastatic cutaneous malignant melanoma: A prospective study of intralesional bacillus Calmette-Guerin versus intralesional DNCB. Cancer (in press).

Javadpour, N.: Current concepts in management of testicular cancer. Urol. Surg. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03807-04 SURG

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Immunoevaluation of Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. B. Chretien	Assistant Chief, Surg. Br.	SURG NCI
Other:	S. D. Lipson	Clinical Associate	SURG NCI
	A. M. Baskies	Clinical Associate	SURG NCI
	E. E. Trahan	Bio. Lab. Tech. (Biochem.)	SURG NCI
	J. R. Castle	Bio. Lab. Tech. (Micro.)	SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Tumor Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 4.5	PROFESSIONAL: 3.0	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In patients with solid malignancies, serum levels of acute-phase proteins (APP) correlated inversely with delayed cutaneous hypersensitivity (DCH) to dinitrochlorobenzene (DNCB) and serum levels of prealbumin and α_2HS-glycoprotein. Serum levels of prealbumin correlated directly with DCH to DNCB. Serum α_2 glycoprotein levels correlate directly with DCH to DNCB and lymphocyte reactivity to phytohemagglutinin (PHA). In patients with small cell carcinoma of the lung receiving intensive chemotherapy also randomized to receive placebo or thymosin twice weekly during induction chemotherapy, increased survival occurred in patients receiving thymosin who had low pretreatment T-cell and α_2HS-glycoprotein levels. A method for determining percentages of human T- and B-cell lymphocyte subpopulations by cytofluorographic analysis (CFGA) and display of data on a computer-generated three-dimensional grid was developed. The percentages of T and B cells plus specimens from normals when analyzed by CFGA did not differ from the results obtained by light microscopy counting.

Serum levels of acute-phase proteins (APP) (α_1-acid glycoprotein, α_1-antitrypsin, haptoglobin, and C-reactive protein), and α_2HS-glycoprotein levels (collectively designated as IRP), were correlated with tumor extent and cellular immunity by correlating their levels with quantitative delayed cutaneous hypersensitivity (DCH) to dinitrochlorobenzene (DNCB) and lymphocyte in vitro reactivity (LR) to phytohemagglutinin (PHA) in 159 patients with operable solid malignancies. Tumor extent was classified by pathologic staging as confined to the primary site or regionally metastatic. All results described were significant at $p<.001-.05$. Levels of α_2HS-glycoprotein correlated directly with LR to PHA. When the patients were grouped by tumor extent, IRP levels correlated with DCH to DNCB. The results show that α_2HS-glycoproteins and APP correlate with immune reactivity in cancer patients.

To determine the relation between parameters of cellular immunity and response to thymosin in vivo we measured the pretreatment peripheral blood T-cell levels and serum α_2HS-glycoprotein levels in patients receiving intensive chemotherapy for small cell carcinoma of the lung. In a trial conducted by the NCI-VA Medical Oncology Branch, 55 patients who received chemotherapy were further randomized to receive either placebo, thymosin 20 mg/m^2, or thymosin 60 mg/m^2 twice weekly.

By life-table analysis of survival, the patients who received chemotherapy and thymosin 60 mg/m^2 had a significantly prolonged survival when compared to the patients treated with chemotherapy alone ($p<.01$). For those with high pretreatment total T-cell or high α_2HS-glycoprotein levels, survival in patients treated with thymosin 60 mg/m^2 was not significantly longer than in those receiving placebo; however, among the patients with low pretreatment total T-cell or α_2HS-glycoprotein levels, those receiving thymosin 60 mg/m^2 had significantly enhanced survival in comparison to patients receiving placebo. Thus, it appears that the association between survival and type of treatment was related to pretreatment total T-cell or α_2HS-glycoprotein levels in patients receiving thymosin in vivo during treatment with chemotherapy for small cell carcinoma of the lung.

A method was developed for determining percentages of human T- and B-cell lymphocyte subpopulations by cytofluorographic analysis (CFGA) and display of the data on a computer-generated three-dimensional grid. Lymphocytes were depleted of either B, T, or both B and T cells by rosetting with erythrocytes and separated by centrifugation. The B-cell and T-cell depleted and non-rosetting cell subpopulations localized on constant, distinct areas of the display grid. The percentages of T and B cells in peripheral blood samples from six normals analyzed by CFGA did not differ from the results obtained by light microscopy counting (LMC). The findings suggest potential value of this methodology for study of lymphocyte subpopulations.

Publications:

Kenady, D.E., Chretien, P.B., Potvin, C., and Simon, R.M.: Thymosin reconstitution of T cell deficits in vitro in cancer patients. Cancer 39: 575-580, 1977.

Kenady, D.E., Potvin, C., Simon, R.M., and Chretien, P.B.: In vitro effect of thymosin on T-cell levels in cancer patients receiving radiation therapy. In Chirigos, M.A. (Ed.): Control of Neoplasia by Modulation of the Immune System. New York, Raven Press, 1977, pp. 305-313.

Kenady, D.E., Chretien, P.B., Potvin, C., Simon, R.M., Alexander, J.D.,Jr., and Goldstein, A.L.: Effect of thymosin in vitro on T cell levels during radiation therapy. Correlations with radiation portal and initial T cell levels. Cancer 39: 642-652, 1977.

Goldberg, N.H., Lipson, S.D., Kenady, D.E., Simon, R.M., Cohen, M.H., and Chretien, P.B.: T-cell levels and response to thymosin in vitro during intensive chemotherapy in cancer patients receiving thymosin. Surg. Forum 28: 151-152, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03811-04 SURG

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Immunotherapy of Animal and Human Sarcomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Rosenberg	Chief of Surgery, NCI	SURG NCI
Other:	W. P. Thorpe	Clinical Associate	SURG NCI
	E. H. Austin	Clinical Associate	SURG NCI
	D. J. Mathisen	Clinical Associate	SURG NCI
	G. H. Mundinger	Clinical Associate	SURG NCI
	J. Brown	Staff Fellow	SURG NCI
	N. Wolmark	Expert	SURG NCI
	S. Schwarz	Bio. Lab. Tech.	SURG NCI
	H. Anding	Bio. Lab. Tech.	SURG NCI
	H. Wexler	Biologist	SURG NCI
	P. Spiess	Biologist	SURG NCI
	C. Hyatt	Biologist	SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.5	5.0	3.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Detailed studies of tumor-host immune interactions in animals and humans with sarcomas are being performed in an attempt to develop new immunodiagnostic and immunotherapeutic techniques for diagnosis and treatment of these tumors. Immune response to murine sarcomas has been extensively evaluated and both tumor-specific and fetal antigens have been identified. Fetal antigens have been identified on human osteogenic sarcomas and these antigens are now being characterized. Attempts are being made to develop adoptive immunotherapeutic techniques utilizing transfer of cells from xenogeneic species to humans with cancer.

Publications:

Parker, G.A., Hyatt, C., and Rosenberg, S.A.: Normal adult murine cells in tissue culture express fetal antigens. Transplantation 23: 161-163, 1977.

Leight, G.S., Sachs, D.H., Williams, G.M., and Rosenberg, S.A.: Transplantation in miniature swine. Transpl. Proc. 9: 575-577, 1977.

Rosenberg, S.A., and Terry, W.D.: Passive immunotherapy of cancer in animals and man. Adv. in Cancer Res. 25: 323-388, 1977.

Parker, G.A., and Rosenberg, S.A.: Cross-reacting antigens in chemically induced sarcomas are fetal determinants. J. Immunol. 118: 1590-1594, 1977.

Rosenberg, S.A.: Lysis of human normal and sarcoma cells in tissue culture by normal human serum: Implications for experiments in human tumor immunology. J. Nat. Cancer Inst. 58: 1233-1238, 1977.

Parker, G.A., and Rosenberg, S.A.: Serologic identification of multiple tumor-associated antigens on murine sarcomas. J. Nat. Cancer Inst. 58: 1303-1309, 1977.

Rosenberg, S.A., Schwarz, S., Anding, H., Hyatt, C., and Williams, G.M.: Comparison of multiple assays for detecting human antibodies directed against surface antigens on normal and malignant human tissue culture cells. J. Immunol. Meth. 17: 225-239, 1977.

Thorpe, W.P., Parker, G.A., and Rosenberg, S.A.: Expression of fetal antigens by normal human skin cells grown in tissue culture. J. Immunol. 119: 818-823, 1977.

Rosenberg, S.A., Parker, G.A., and Thorpe, W.P.: Expression of oncofetal antigens by murine and human normal cells in tissue culture. Israel J. Med. Sci. 14: 98-104, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03812-04 SURG

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies with a Chemically-Induced Rat Colon Cancer Model

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. R. Baker	Senior Investigator	SURG	NCI
Other:	D. F. Devereux	Clinical Associate	SURG	NCI
	J. M. Ward	Acting Ch., Tumor Pathol. Br.	TP	NCI
	S. F. Stinson	Pathologist	TP	NCI
	R. M. Simon	Ch., Biometric Res. Br.	DCT	NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

DMH-induced rat colon cancer is being explored as a model of human colorectal cancer.

PHS-6040
(Rev. 10-76)

Recently completed studies emphasize the importance of gut transit time/stool bulk as significant variables in the pathogenesis of these chemically-induced tumors. Rats maintained on liquid Vivonex, a low residue - elemental diet, during the induction period have a twofold prolongation in their gut transit time, a tenfold reduction in stool weight, and develop twice as many tumors as compared with either rats fed the same elemental diet admixed with non-nutritive bulk (cookies) or lab chow.

In a separate study the use of intravenously administered Cr^{51} tagged red blood cells has been explored for noninvasively detecting colorectal tumors. It appears both sensitive and accurate as a test. Rats bearing colon tumors shed in their stool between 0.087-1.8 ml of blood per day. The test has accurately identified 21 rats with gut tumors and 43 rats without gut tumors. One false positive and 2 false negatives have been noted.

Studies are planned in the carcinogenesis area to examine stool flora in rats given DMH while fed liquid Vivonex, cookies, and chow. In the tumor therapy area, studies will be done to compare treatment of the primary colon tumors by surgical resection and intralesional immunotherapy, the latter being a relevant adjunctive approach in man. Efforts are in progress to define a CEA-like material in this rat system.

The magnitude of the colorectal cancer problem in man makes this animal model very attractive as a focus for studying pertinent clinical questions.

Publications:

Baker, A.R., Ward, J.M., Simon, R.M., Stinson, S.F., Devereux, D.F.: Gut transit time is an important factor in the pathogenesis of DMH induced rat colon cancer. Surg. Forum (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03826-04 SURG

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biologic Tumor Markers in the Cells and Sera of Patients with Urologic Cancers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Javadpour	Senior Investigator	SURG NCI
Other:	R. C. Emmott	Guest Worker	SURG NCI
	E. A. Woltering	Clinical Associate	SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Techniques have been developed to localize and quantitate biologic tumor markers in the cells and sera of patients with urologic cancers. These markers include human chorionic gonadotropin, alphafetoprotein, ABO(H) antigens, and steroid receptors. These markers have been correlated with the histology, grade, stage, and management of patients with urologic cancers.

Publications:

Scardino, P.T., Bergman, S., Terrill, R., and Javadpour, N.: Countercurrent exchange of testosterone in the primate spermatic cord. Surg. Forum 27: 579-581, 1976.

Javadpour, N., and Scardino, P.T.: Recent advances in the immunobiology of genitourinary cancer. Urology 9: 377-384, 1977.

Scardino, P.T., Cox, H.D., Waldmann, T.A., McIntire, K.R., Mittemeyer, B., and Javadpour, N.: The value of serum tumor markers in the staging and prognosis of germ cell tumors of the testis. J. Urol. 118: 994-999, 1977.

Bergman, S., and Javadpour, N.: The cell surface antigen A, B or O(H) as an indicator of malignant potential in Stage A bladder carcinoma: Preliminary report. J. Urol. 119: 49-51, 1978.

Javadpour, N., Hyatt, C.L., and Barone, J.J.: Common antigens found on fetal cells and viral transformed adult prostatic tissue. J. Surg. Oncol. 10: 245-250, 1978.

Kurman, R.J., Waldmann, T.A., and Javadpour, N.: Cellular localization of alphafetoprotein and human chorionic gonadotropin in germ cell tumors of testis utilizing an indirect immunoperoxidase technique. A new approach to classification utilizing tumor markers. Cancer 40: 2136, 1977.

Emmott, C., Javadpour, N., Bergman, S., and Soares, T.: Correlation of the cell surface antigens with stage and grade in cancer of the bladder. J. Urol. (in press).

Javadpour, N., Scardino, P.T., and Bergman, S.: The role of the radioimmunoassay of serum alphafetoprotein and human chorionic gonadotropin in intensive chemotherapy and surgery of the metastatic testicular tumor. J. Urol. (in press).

Javadpour, N., Waldmann, T.A., and McIntire, K.R.: The role of human chorionic gonadotropin and alphafetoprotein in sera and tumor cells of patients with seminoma. Cancer (in press).

Javadpour, N.: Immunochemical detection of human chorionic gonadotropin and alphafetoprotein in human testicular tumor. Nat. Cancer Inst. Monogr. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06652-02 SURG

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Decreasing Strengths of Cell-Mediated Immune Responses Generated Following Repeated Alloantigenic Stimulation in Inbred Strains of Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. H. Sugarbaker	Senior Investigator	SURG NCI
Other:	A. E. Chang	Clinical Associate	SURG NCI
	W. Matthews, Jr.	Research Associate	SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2.8	PROFESSIONAL: 1.8	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although cell-mediated immune responses to a variety of alloantigenic stimuli have been studied thoroughly in the past, responses that occur after prior alloantigenic stimulation have not been previously thoroughly investigated. Our studies involve an assessment of cell-mediated immune responses after the host has responded to a variety of alloantigenic stimulation to the same and other antigens. We find a marked specific decrease in the generation of cytotoxic cells after previous immunizations. The diminished response is largely specific for the immunizing alloantigen. Mechanisms involved in this suppression include elimination of graft proliferation in immunized hosts, elimination of stimulator cells by cytotoxic cells, and depression by alloantisera. The significance of this work comes from attempts to understand regulatory mechanisms of cellular immune responses.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06653-02 SURG

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Metabolic Studies in Tumor-Bearing Patients and Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. F. Brennan	Head, Sec. of Surg. Metab.	SURG NCI
Other:	S. F. Lowry	Clinical Associate	SURG NCI
	J. A. Norton	Clinical Associate	SURG NCI
	D. M. Crnic	Clinical Associate	SURG NCI
	M. Maher	Clinical Nurse	SURG NCI
	D. F. Devereux	Clinical Associate	SURG NCI
	M. Popp	I.P.A., Univ. of Cincinnati	SURG NCI
	M. E. Burt	Clinical Associate	SURG NCI
	D. White	Computer Analyst	SURG NCI
	C. Gorschboth	Medical Technologist	SURG NCI
	D. Jones	Biologist	SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Surgical Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10.0	7.0	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgery Branch is vigorously involved in the assessment of the efficacy of total parenteral nutrition as a means of nutritional support of the cancer-bearing host. These studies are directed at utilizing new methodologies employing stable isotopes and labelled substrates combined with standard assessment of nitrogen balance and 3-methylhistidine excretion as determinants of host-protein preservation. In addition, two prospective randomized studies have been initiated as mentioned under 03801-08. Within the lab, attempts to nutritionally deprive human tissue culture cell lines of essential amino acids are continuing. The interest in animal models of protein depletion and methods for preservation of host tissue by animal TPN, exercise and steroids continue.

PHS-6040
Rev. 10-76

Publications:

Reilly, J.J., Goodgame, J.T., Jones, D.C., and Brennan, M.F.: DNA synthesis in rat sarcoma and liver: The effect of starvation. J. Surg. Res. 22: 281-286, 1977.

Brennan, M.F.: Uncomplicated starvation versus cancer cachexia. Cancer Res. 37: 2359-2364, 1977.

Brennan, M.F.: Tumor versus host metabolism. Cancer Bulletin (M. D. Anderson) (in press).

Goodgame, J.T., Lowry, S.F., and Brennan, M.F.: Essential fatty acid deficiency in total parenteral nutrition: Time course of development and suggestions for therapy. Surgery (in press).

Lowry, S.F., Goodgame, J.T., Maher, M.M., and Brennan, M.F.: Parenteral vitamin requirements during intravenous feeding. Am. J. Clin. Nutr. (in press).

Lowry, S.F., Goodgame, J.T., Smith, J.C., Maher, M.M., Makuch, R.W., Henkin, R.I., and Brennan, M.F.: Deficiencies of zinc and copper trace elements during total parenteral nutrition. Ann. Surg. (in press).

McCarthy, D.M., May, R.J., Maher, M., and Brennan, M.F.: Trace metal and essential fatty acid deficiency during total parenteral nutrition. Am. J. Dig. Dis. (in press).

Goodgame, J.T., Lowry, S.F., and Brennan, M.F.: Body weight change and nutritional adequacy in the parenterally alimented rat. J. Surg. Res. (in press).

Devereux, D.F., Thibault, L., Boretos, J., and Brennan, M.F.: The quantitative and qualitative impairment of wound healing by adriamycin. Cancer (in press).

Bagley, D.H., McLowry, J., Beazley, R.M., Gorschboth, C., and Ketcham, A.S.: Antibiotic concentration in human wound fluid after intravenous administration. Ann. Surg. (in press).

Brennan, M.F., and Goodgame, T.: Essential fatty acid deficiency in total parenteral nutrition: Time course of development and suggestions for therapy. Surgery (in press).

Goodgame, J.T., Pizzo, P., and Brennan, M.F.: Iatrogenic lactic acidosis: Association with hypertonic glucose administration in a patient with cancer. Cancer (in press).

Lowry, S.F., Goodgame, J.T., Norton, J.A., Jones, D.C., and Brennan, M.F.: The effect of protein malnutrition on host and tumor composition. Surg. Forum (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06654-01 SURG

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Immune Studies in Malignant Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. F. Sindelar	Senior Investigator	SURG NCI
Other:	None		

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with sarcomas are studied for evidence of serum reactivity against tumor-specific determinants expressed on cultured syngeneic tumor cells using techniques of immunofluorescent staining. Roles of tissue-specific xenogeneic antibodies are investigated for possible approaches to developing method of immunotherapy in a carcinogen-induced pancreatic carcinoma in hamsters.

PHS-6040
(Rev. 10-76)

SUMMARY REPORT
Associate Director for the Baltimore Cancer Research Program
Division of Cancer Treatment
National Cancer Institute
Baltimore, Maryland
October 1, 1977 through September 30, 1978

The Baltimore Cancer Research Program has completed its 4th year since transferring to the University of Maryland Hospital. The Clinical Branch, including the support services of the Clinical Research Pharmacy Service, Microbiology Section, Infection Research Section, and Cell Component Therapy Unit, has produced a number of fruitful projects. During the reporting interval, care has been provided for approximately 10,300 inpatients days and 13,200 outpatient visits.

The Laboratory of Clinical Biochemistry and Laboratory of Molecular Biology will move from facilities at the Baltimore Public Health Service Hospital to our new laboratory facilities at the University of Maryland Hospital this summer. Shortly thereafter, construction of a new, modern and efficient outpatient facility, and a laminar air flow intensive care unit will begin.

The Clinical Branch continues to pursue Phase I and II drug testing. New drugs, such as AMSA and PALA are nearing completion of Phase I testing, and a Phase II study of anguidine in colon cancer is also nearing completion. The Phase II evaluation of maytansine for squamous cell carcinoma of the lung is underway. In addition, a Phase II study of cis-dichlorodiamminplatinum in genito-urinary cancer is well under way.

A Phase I study of hyperthermia using equipment supplied by the National Aeronautics and Space Aministration has been completed. The results indicate that patients can tolerate 4 hours of exposure to 41.8 degrees centigrade, a temperature associated in other anecdotal reports in the literature with favorable responses in cancer patients. A Phase III study of hyperthermia and adriamycin for patients with metastatic sarcoma is planned and will begin shortly.

Phase III studies of daunorubicin or adriamycin and cytosine arabinoside in adult acute nonlymphocytic leukemia have demonstrated a 70% complete response rate. Current studies in adult acute leukemia are evaluating various maintenance schemes, including late intensification therapy, splenectomy, and immunotherapy with neuramininase treated leukemic cells. A study of streptozotocin, cyclophosphamide, adriamycin and bleomycin for advanced previously untreated Hodgkin's disease has indicated a high degree of activity for this combination. Cytoxan, adriamycin and VP16-213 has produced a 60% complete response rate in patients with oat cell carcinoma of the lung. Combined modality studies of Hodgkin's disease continue to show significant advantage for radiotherapy-chemotherapy in patients with stage IIIA or with early stages associated with "E" substage of the lung. Early evaluation of a new Hodgkin's disease study comparing chemotherapy with radiotherapy plus chemotherapy shows that MOPP chemotherapy and radiotherapy are equally effective in producing complete responses in patients with stage I-IIIA Hodgkin's disease.

Programs of the Section of Infection Research and Research Microbiology have continued in five major areas, namely surveillance, epidemiology, diagnosis, treatment, and prevention. This work has led to the identification of refined empiric antibiotic combinations with which to treat febrile granulocytopenic cancer patients more and more successfully.

Studies with autologous frozen platelets in acute leukemia patients without compatible donors have shown them to be life saving. This study, plus the prophylactic granulocyte transfusion study represent major contributions from our Cell Component

Therapy Unit to our Program and to the Treatment of Leukemia and cancer patients at large.

The Laboratory of Clinical Biochemistry general areas of investigation include cellular control mechanisms; cellular organelle ultrastructure and function; drug disposition, metabolism and mechanism of action; and the study of tumor biology and immunology. Current studies are directed at identifying and defining the mechanisms by which endogenous and exogenous substances modulate cell proliferation and differentiation. The Branch has investigated new pathways for drug metabolism such as the enzymatic pathways for aldehyde and ketone reduction. Pharmacologic studies range from pharmacokinetics and drug metabolism in patients treated on Clinical Oncology Branch protocols to the study of molecular interactions between drugs and macromolecules. A major research effort is directed at defining mechanisms of action of the anthracycine antibiotics and other cytotoxic antibiotics. Analytical techniques for drugs and drug metabolites which can then be applied to clinical pharmacologic problems have been developed. Close integration of fundamental laboratory research and clinical studies is a standard working approach of this laboratory. Clinical pharmacologic studies are carried out in collaboration with the Clinical Oncology Branch BCRP to determine effective chemotherapeutic regimens for cancer therapy.

One major area of concentration within the Laboratory of Molecular Biology has been the study of cellular regulation particularly as it effects those factors which control the stabilization of messenger RNA (mRNA) in normal and neoplastic tissues. The Laboratory is continuing its study of the relationship between oncogenic viral proteins and cell transformation. It has been determined that p30 and reverse transcriptase can be isolated from Rauscher leukemia virus as a complex and that the complex is present in the thymus and spleen of AKR mice at the onset of leukemia. The Laboratory has isolated and purified other proteins within the virion core (that is p10, p12, and p15) in an effort to study more completely viral assembly and its relation to oncogenesis.

The Laboratory is dependent on clinical material from patients treated on Clinical Oncology Branch protocols for much of this laboratory work.

In the last year, a closer scientific interaction among the various DCT branches in Bethesda and Baltimore has taken place. To date, this cooperation has led to joint lymphoma and leukemia protocols and to joint Phase I studies of drugs considered of prime importance such as PALA. This cooperation has led to better utilization of clinical material on both campusses and to faster clinical results derived from larger numbers of patients that previously was not possible for the DCT intramural program.

SUMMARY REPORT
CLINICAL ONCOLOGY BRANCH
BALTIMORE CANCER RESEARCH PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE
October 1, 1977 through September 30, 1978

The Branch has undertaken a number of interesting projects in the last reporting period and has completed some valuable studies outlined in the previous report. A Phase I study of nitrous oxide is progressing in acute nonlymphocytic leukemia. Additional Phase I studies currently in force include a study of AMSA nearing completion, and a study of PALA, just begun in collaboration with the Clinical Center and the NCI-VA unit. Phase II studies of maytansine in lung cancer and anguidine in colon cancer are nearing completion. Phase II studies of dianhydrogalactitol and piperazinedione in brain tumors have been reported. In addition, a Phase II study of azotomycin in metastatic sarcoma has been completed. A Phase III study of methotrexate, platinum, and bleomycin in head and neck cancer has been completed and submitted for publication. Phase III studies of anthracycline antibiotics in conjunction with cytosine arabinoside in acute nonlymphocytic leukemia have continued and yielded valuable results. Seventy percent of adults achieved a complete remission with these agents and early indications are that late intensification therapy significantly prolongs remission duration in adult acute nonlymphocytic leukemia.

Combined modality studies of Hodgkin's disease continue to show significant advantage for radiotherapy-chemotherapy in patients with Stage IIIA or Stage II_E. A new study of Hodgkin's disease early stages comparing radiotherapy-chemotherapy with chemotherapy alone shows that both regimens are equally productive of complete remissions. Phase III studies in oat cell carcinoma of the lung have produced some of the best data obtained with chemotherapy alone. In fact, the data is comparable for early stage disease protocols utilizing a combination of radiotherapy and chemotherapy.

In the Cell Component Therapy area, studies of frozen autologous platelet transfusions for acute leukemia patients continue to be rewarding. We have transfused platelets, frozen at liquid nitrogen temperatures, for up to two years back to the donor at a time when he was thorombocytopenic in association with leukemic relapse. The transfusions have yielded increments suggesting approximately a 50% recovery rate of frozen platelets. A prophylactic granulocyte transfusion study is under way and it is too early for evaluation.

Programs of the Sections of Infection Research and Research Microbiology involve five major areas: surveillance, epidemiology, diagnosis, treatment, and prevention. The epidemiology of Aspergillus infections in the granulocytopenic patient with acute leukemia was further defined to indicate the importance of prior broad spectrum antimicrobial therapy, especially when it causes suppression of normal microbial growth in the nose at a time when air is contaminated with spores of Aspergillus. Diagnosis of pneumonia by sequential approach of transtracheal aspiration followed by transtracheal bronchial brush biopsy will establish an etiologic diagnosis in approximately 75% of patients with pneumonia in whom sputum evaluations are nondiagnostic. Treatment studies have included evaluations of new antimicrobials, both in the laboratory and in the clinic. Two prospective, randomized empiric antibiotic therapy trials have shown that the combination of ticarcillin plus an aminoglycoside has an excellent therapeutic index. New drugs, including piperacillin and mezlocillin, have been evaluated in vitro and found to be superior to any of the semisynthetic penicillins heretofor available, especially with regard to gram negative bacilli. They will therefore shortly enter clinical trial.

Infection prevention has emphasized refinements of antimicrobial prophylaxis. The usual combinations of gentamicin, vancomycin, and nystatin was compared to gentamicin and nystatin and the results are that the gentamicin-nystatin regimen was comparable to the larger combination in infection prevention. The elimination of vancomycin removes the most offensive tasting antibiotic from the combination and reduces the cost of prophylactic oral antibiotic treatment by 20%.

Biologic markers of malignant disease such as terminal transferase and reverse transcriptase have been studied in some detail and the data correlated with the type and extent of hematologic malignancy.

The massive research data accumulated by the Branch has been prepared for analysis on the Promis System. Physicians, nurses, pharmacists, and other related health care personnel take part in the input and the retrieval of this data.

Participation in oncology programs at the two medical schools in Baltimore and at other hospitals around the community has provided a source of patient material for our studies and has enabled us to bring to the practicing and academic physician in the community, the impact of the therapeutic advances resulting from the BCRP program.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06230-09 COB

PERIOD COVERED
October 1, 1977 through Sept.30, 1978

TITLE OF PROJECT (80 characters or less)

Diagnostic Laparotomy and Splenectomy for Staging Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	Charles H. Diggs, M.D.	Senior Investigator	COB NCI
Others:	John Sutherland, M.D.	Pathology, Univ. of MD Hospital	
	James Ryan, M.D.	Nuclear Medicine, Univ. of MD Hospital	

COOPERATING UNITS (if any)

Pathology, Tumor Surgery, Radiology and Nuclear Medicine Departments,
University of Maryland Hospital, Baltimore, Maryland

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of this study have been to determine the value of staging laparotomy on evaluating the accuracy of the extent of abdominal disease at the time of initial evaluation and what influence this has on the initial therapy for patients with early stage Hodgkin's disease. 175 patients with Hodgkin's disease have undergone staging laparotomy and splenectomy. This has revealed that the pre-operative assessment of the extent of abdominal involvement was incorrect in approximately 40% of these cases. The prediction of splenic involvement by Hodgkin's disease was incorrect in one-third of the patients and approximately one-third of abnormal lymphangiograms could not be confirmed at laparotomy. A certain number of cases of involvement of nodes in the porta hepatic region outside the usual irradiation therapy ports were discovered. A small number of patients were also found to have documented liver involvement which had not been suspected by pre-operative evaluation. These findings have altered the therapeutic approaches significantly in a number of patients. The results of these studies have been important in the subsequent design of further therapeutic protocols for the treatment of Stages I-A to III-A Hodgkin's disease.

Accurate determination of extent of lymphomatous involvement with Hodgkin's disease is important for the individual patient's prognosis and therapy. Valid assessment of the efficacy of different therapeutic modalities used in a population with Hodgkin's disease is also dependent upon a precise localization of disease prior to therapy. The purpose of this study is to determine more accurately the extent of abdominal disease at the time of diagnosis and prior to therapy by performing diagnostic laparotomy.

Analysis of 175 patients with Hodgkin's disease who have undergone staging laparotomy and splenectomy has revealed that pre-operative assessment of the extent of abdominal involvement was incorrect in approximately 40% of the cases. Prediction of splenic involvement by Hodgkin's disease was incorrect in 1/3 of the patients, approximately 1/3 of abnormal lymphangiograms could not be confirmed at laparotomy, and a number of cases of involvement of nodes in the porta hepatis region outside the usual radiation therapy ports were discovered. Occult hepatic involvement was discovered in some cases in which liver function tests and physical examination were normal, and others suspected of having liver involvement were shown to have disease confined only to lymph nodes. Theses findings altered therapeutic approaches significantly in many patients.

It was discovered that patients presenting with supradiaphragmatic Hodgkin's disease had occult disease limited to the spleen, splenic hilum and celiac axis nodes in most cases in which abdominal tumor was documented at laparotomy. Extranodal involvement was not seen in any of the 85 patients with supradiaphragmatic clinical Stage I or II Hodgkin's disease and most patients would have had all apparent disease adequately treated had extended field radiation therapy been administered without performing staging laparotomy. Therefore, a subsequent project analyzed the value of performing staging laparotomy in patients with early clinical stage Hodgkin's disease. This present study is now closed to patient accrual.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06259-06 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Extension of the Computerized PROMIS System into the Cancer Treatment Area

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Esterhay, M.D.	Senior Investigator	COB NCI
Others:	Paul Chang, M.D.	Senior Investigator	COB NCI
	Clarence Fortner	Pharmacist	COB NCI
	William Robinson	Computer Specialist	COB NCI
	Joann Gustafson	Computer Analyst	COB NCI
	Charles Schiffer	Head, Cell Component Therapy Unit	COB NCI
	Steve Schimpff	Head, Infection Research Section	COB NCI
	Viola Mae Young, Ph.D.	Head, Microbiology Section	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch and Acting Director BCRP	COB NCI

COOPERATING UNITS (if any)
PROMIS Laboratory, Department of Medicine, University of Vermont, Burlington, Vermont

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.5	6.5	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Baltimore Cancer Research Program (BCRP) has developed cancer guidance displays (treatment protocols) on the computerized Problem Oriented Medical Information System (PROMIS) in Burlington, Vermont at the University of Vermont School of Medicine, with the aid of a communication linkage between BCRP and PROMIS. The computerized displays for the logic and action of treating cancer problems are defined by the creation and sequencing of the computer display pages or frames. These displays provide an automatic connective device between the four phases of medical action of the Problem Oriented Medical Record. The problem defining sequence determines the specific plan and it is that plan which is in part a protocol or series of protocols for treating a specific cancer problem. The protocol couples the health care provider to the protocol specific antineoplastic drug displays which provide feed back loops to the patient's problem list and outstanding orders. It is through the use of these explicit informational and directional displays that the health care provider can couple learning with doing while gathering information and treating patients' cancer problems.

To extend the Computerized Problem-Oriented Medical Information System (PROMIS) to the cancer treatment area to allow the development of structured input ("cancer displays") for coupling learning with doing for all personnel who care for individual cancer patients entered on problem specific plans (cancer treatment research protocols).

Methods Employed:

1. The computer terminal being used for this purpose is a touch-sensitive cathode ray tube display device similar to those in use in Burlington, Vermont, where the computer is located. BCRP has been responsible for the installation since February, 1972. All programming and operation of the computer system have been done at the University of Vermont.

2. The basic components of the remote terminal at the BCRP are the same as the local terminals in Burlington, Vermont: display screen, display memory, keyboard, touch screen and communication link to the computer in the PROMIS Laboratory, Burlington, Vermont.

Results:

1. The BCRP staff has developed anti-neoplastic drug displays which have been integrated with the displays already on file in Burlington, Vermont, as part of the PROMIS system. The drug information sequences have been up-dated and new sequences have been added. Bulsulfan drug displays have been completed and audited. A brief drug summary for thiotepa is in progress. Update and revision of the pyrimidine antagonists are in progress. Reference citations using new citation tables are complete for alkylating agents and are in progress for antimetabolites as they are being updated. Monitoring information for all antineoplastic agents is continuing. All alkylating agents have been audited and updated. Drug dosage information structures were expanded to include: 1) general information for planning drug dosages; 2) technique to prepare drugs and 3) technique to administer drugs.

2. Problem formulation sequences for breast cancer and lung cancer were revised and expanded to match current standard terminology. Lymphoma problem formulation sequences were updated to match current standards. Problem formulation sequences for colon cancer, sarcomas, ovarian, testicular and melanoma will be updated as well.

3. Problem specific plans for small cell carcinoma of the lung have been completed including BCRP protocol #7705 which will serve as a model for other protocols that will be updated and added.

4. Bone marrow reporting has been completed. Definitions of cell types will be added in the near future.

5. Thorax autopsy reporting has been completed. Additional reporting sequences for other organ systems are in progress.

The computerized displays for the logic and action of treating cancer problems are defined by the creation and sequencing of the computer display pages or frames. These displays provide an automatic connective device between the four phases of medical action of the Problem-Oriented Medical Record. The problem formulation sequence determines the specific plan and it is that plan which is in part a protocol or series of protocols for treating a specific cancer problem. The protocol couples the user to the protocol specific anti-neoplastic drug displays which provide feedback loops to the patient's problem list and outstanding orders. It is through the use of these explicit informational and directional displays that the user is provided guidance while gathering information and treating patients' cancer problems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06263-07 COB

PERIOD COVERED
October 1, 1977 through Sept. 30, 1978

TITLE OF PROJECT (80 characters or less)

Protocol for Staging and Treatment of Lymphosarcoma and Reticulum Cell Sarcoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Senior Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
Others:	Robert Slawson, M.D.	Division Radiation Therapy, University of Maryland Hospital	

COOPERATING UNITS (if any)

Radiotherapy Service and Surgical Service,
University of Maryland Hospital, Baltimore, Maryland 21201

LAB/BRANCH
Clinical Oncology Branch

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NCI, NIH Baltimore, Maryland 21201

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

At the time of presentation, non-Hodgkin's lymphomas are frequently disseminated. There exists, however, a number of patients with localized disease following completion of all clinical and pathologic staging procedures. It is in this group of patients that radiotherapy is most useful. However, the question of whether adjuvant chemotherapy following radiation therapy is of any benefit is as yet unanswered. Patients who are found to be pathologically Stage I and II non-Hodgkin's lymphomas following a variety of staging procedures including staging laparatomy, are randomized to receive either extended field irradiation alone, or extended field irradiation followed by six courses of chemotherapy using Cytoxan, vincristine and prednisone. Thirteen patients whose staging laparotomies were entirely negative have been randomized to one or the other of the treatment arms. Too few patients have been accrued at this time to determine the value of adjuvant chemotherapy in this setting.

1. To assess the value of surgical staging in patients with apparently localized non-Hodgkin's lymphomas.

2. To determine whether adjuvant chemotherapy will prolong remissions in patients with pathologically localized lymphomas treated with radiation therapy.

Patients with malignant non-Hodgkin's lymphomas will undergo a variety of staging procedures. Those who remained clinically localized following non-invasive procedures will undergo staging laparotomy to detect occult foci of intra-abdominal disease. Patients who are found to be pathologic Stage I or II will then be randomized to receive (a) radiation therapy alone, or (b) radiation therapy followed by six courses of combination chemotherapy using Cytoxan, vincristine and prednisone. Patients who are found to have advanced disease by these staging procedures will be treated with chemotherapy alone on a different regimen. Of the patients undergoing all staging procedures, there have been 13 who have been found to have pathologic Stage I or II. At this time, there are too few patients in either of the study arms to determine the value of adjuvant chemotherapy.

Staging laparotomy as an additional diagnostic test to determine the extent of disease in the non-Hodgkin's lymphomas should allow better patient selection for attempts at curative radiation therapy in those patients who are found to have truly localized disease. Adjuvant chemotherapy conceivably may offer prolonged disease-free and overall survival.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06275-09 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Cell Transfusion Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles A. Schiffer, M.D.	Senior Investigator	COB NCI
	Joseph Aisner, M.D.	Senior Investigator	COB NCI
	Peter A. Daly, M.D.	Visiting Associate	COB NCI
	Peter H. Wiernik, M.D.	Chief,Clinical Oncology Branch	COB NCI
Other:	Karen Lichtenfeld, M.D.	Clinical Associate	COB NCI
	Martin Helrich, M.D.		
	Lucy Barrett, Ph.D.		
	Michael Loberg, Ph.D.		

COOPERATING UNITS (if any)
Community Blood and Plasma Service, Baltimore; Dr. Paul Terasaki, University of California, Los Angeles; the University of Maryland, Departments of Pathology, Anesthesiology and Nuclear Medicine

LAB/BRANCH
Clinical Oncology Branch

SECTION
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INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

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8.5	4.5	4.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the past year, our HLA typed donor file has been expanded and histocompatible platelets and granulocytes are now available for both therapeutic and prophylactic transfusions. Preliminary results of a prophylactic granulocyte trial indicate a decreased rate of infection in the granulocyte transfused group although the rate of alloimmunization may be increased. Autologous frozen platelets have become a major part of our support program and analysis of 91 transfusions to 25 leukemic patients demonstrates recoveries which are 67% of results with fresh platelets. Cell Collection studies have demonstrated that premedication with dexamethasone does not adversely affect in vivo and in vitro platelet function and that platelets collected with the Haemonetics Model 30 function and circulate normally. Gravity leukopheresis has been shown to be an efficient but slow method of granulocyte collection perhaps best suited to collection of blasts and leucocytes from patients with chronic myelogenous leukemia. An analysis of approximately 150 patients with acute nonlymphocytic leukemia did not reveal any association between HLA phenotype or haplotype and the occurrence or prognosis of the leukemia. Studies with indium labeled platelets and studies of granulocyte migration to skin chambers are in progress.

Project Description: Z01 CM 06275-09 COB

The Cell Transfusion Program is organized to provide platelets and granulocytes for the treatment and prophylaxis of hemorrhagic and infectious complications in patients with leukemia, lymphoma and other types of solid tumors receiving aggressive therapy. In addition, an active laboratory focusing on various aspects of platelet and granulocyte function is in operation.

I. Platelet Transfusion

Approximately 8500 units of platelet concentrate were transfused in 1977. Most of the platelets transfused were obtained by two unit plateletpheresis of random donors performed by the Community Blood and Plasma Service. Approximately 2,000 units were collected and processed by the Cell Component Therapy Unit and consisted of HLA matched platelets, platelets administered with granulocyte transfusion, and frozen platelets. The transfusion of HLA matched platelets has been expedited by the recruitment at the Community Blood and Plasma service of approximately 700 HLA matched donors which supplements the file of approximately 1,000 donors at the BCRC. The number of HLA matched donors should increase by approximately 1,000/annum and allow studies measuring the immunogenicity of HLA antigens, the use of platelets mismatched for cross-reactive HLA antigens, and an analysis of patterns of development of anti HLA antibody.

As part of a prophylactic granulocyte transfusion study (BCRC 7702 see below) a control group of patients is receiving platelets obtained from a limited number of HLA typed donors. There is little literature available describing the rate of immunization in such patients supported solely with single donor platelets and more information should be acquired from the study. In addition, analysis is in progress of the changes in lymphocytotoxic antibody which occur over a period of time in patients with acute non-lymphocytic leukemia. It appears that some alloimmunized patients actually lose their antibody and do not regain it despite repeated antigen challenge. In particular, patients who have been supported only with autologous frozen or HLA matched platelets have had marked decreases in their antibody levels making subsequent transfusion support simpler. Clinical factors which may influence both primary and anamnestic antibody response progress are being analyzed.

Platelet cyropreservation remains one of the major interests of the unit. Three serial studies of platelet cryopreservation have been done at the BCRC using 5% DMSO as a cryoprotective agent and have been characterized by a progressive simplification of the technique, a decrease in the freeze-thaw loss and significantly improved clinical results. A recent analysis of 91 transfusions to 25 patients with leukemia has been completed and has resulted in 1 hour post-transfusion recoveries which are approximately 2/3 of that to be expected with fresh platelets. The platelets appear to be hemostatically active and autologous frozen platelets now provide the transfusion for support for 40% of our patients with leukemia during maintenance and initial reinduction chemotherapy. Large numbers of patients have been supported using only autologous platelets. The large number of variables which may affect these results are in study in the laboratory. It has been demonstrated that platelets from patients with leukemia in remission aggregate normally and have normal PF-3 release. An analysis of the size of the platelets both pre and post freezing is in progress in an effort to provide an in vitro tool which would correlate with in vivo results and allow systematic modification of various steps in the freezing and thawing process. In addition, studies with a cytograph and an in vitro system measuring enzyme release will also be done in an attempt to improve upon the freezing results.

Using the Model 30 blood processor, platelets are collected along with granulocytes. Because donors are routinely premedicated with dexamethasone in order to increase granulocyte yield, a study of the effects of dexamethasone on platelet function was performed. Normal donors were premedicated with 10 mg of dexamethasone and bleeding times and aggregation studies were performed. There were no significant changes in platelet aggregation or bleeding times following administration of dexamethasone and it was concluded that this drug represents an appropriate additive for granulocyte collection.

As an outgrowth of studies on the effects of local anesthetics on platelet function, a cooperative study with the Department of Anesthesiology was performed which measured the effect of general anesthesia in patients undergoing minor surgical procedures on platelet aggregation. No changes in platelet aggregation were noticed in thirteen patients undergoing a variety of minor operations and receiving standard inhalational anesthetic agents.

In cooperation with the Department of Nuclear Medicine, we have begun to study the feasibility of labeling platelets and granulocytes with ^{111}Indium Oxine. It has been demonstrated that platelets cannot be labeled in a plasma media but successful labeling can be achieved in Tyrodes solution. The technique has worked well in the laboratory and will shortly be utilized to perform platelet kinetic studies post transfusion.

II. Granulocyte Transfusion:

Therapeutic granulocyte transfusions are now administered as early in the course of serious infection as possible, particularly in patients with pneumonia and/or gram negative bacteremia in whom long periods of granulocyte aplasia are anticipated. Joint rounds are now made twice a week with the Infectious Disease Section and formal transfusion rounds are now made once a week. This has resulted in more uniform application of granulocyte transfusion and excellent clinical follow-up.

A study to determine the effect of prophylactic administration of granulocytes is in progress and presently a total of 15 patients have been randomized. There appears to be a decreased rate of acquisition of infection in patients randomized to receive granulocyte transfusion on an every-other-day basis. Obviously, these results are only preliminary. The rate of alloimmunization appears to be higher in the group receiving granulocytes with transfusion reactions and leukoagglutinins developing prior to clinical refractoriness to platelets. Granulocytes for this study are obtained from the Haemonetics Model 30 whereas therapeutic granulocytes are obtained from the Model 30 and filtration leukapheresis techniques. In addition to these techniques, studies of gravity leukapheresis have been carried out in the last year. It was determined that variation of temperature had no effect on the efficiency of collection, but that longer periods of sedimentation increased the yield per unit processed. Granulocytes obtained by this method were normal morphologically and had normal bactericidial capacity. Unfortunately, the yield of granulocytes for transfusion was limited by the small amount of blood which can be processed in a tolerable period of time. Studies using prototype bags provided by the Fenwal Corporation will be carried out shortly in an attempt to determine the precise number of granulocytes which can be obtained in a given period of time. In addition, it is felt that within the next year prototype studies of a new centrifugation apparatus from the Fenwal Corporation will also be carried out.

Because of low post-transfusion recovery and count increments following granulocyte transfusion, it has been difficult to perform reproducible studies measuring the effects of histocompatibility, donor premedication, etc. In an attempt to approach these questions we are presently developing a skin chamber technique and have preliminary data on the reproducibility of the technique in normals. The next stage of the project will be to place skin chambers on patients receiving prophylactic granulocytes and to study the effects of differences in the histocompatibility and collection techniques on granulocyte migrations with inflammatory sites. It is hoped that these studies will eventually be coordinated with Indium-labeled granulocyte transfusion.

III. Other laboratory functions

A. Lymphocyte and blast collection

B. Collection of blasts from patients with leukemia for cryopreservation.

C. Studies of cryopreservation techniques for blasts in progress.

D. Therapeutic plasmapheresis of selected patients.

E. Large scale collection of zoster immune plasma from normal donors recovering from chicken pox or herpes zoster.

IV. Additional Investigations

A. HLA and ANLL: In cooperation with Dr. Paul Terasaki an analysis has been made of approximately 150 adult patients with acute non-lymphocytic leukemia who have been HLA typed at the BCRC and demonstrates that there is no preponderance of any particular HLA phenotype or haplotype. There is no relationship between HLA type, achievement of remission or long term survival.

B. In conjunction with Dr. Schnapper and employing interviewing and questionnaire techniques the psychological reaction of donors and recipients is being studied.

C. In vitro test of bleeding time: An apparatus supplied by the Payton Instrument Company is being studied which has the potential of allowing repeated in vitro assessment of bleeding time thereby obviating the painful and sometimes disfiguring scars associated with the template bleeding time.

D. A comparison of in vivo recovery following transfusion of platelets obtained by either "standard bag techniques" or Haemonetics Model 30 techniques utilizing the same donor recipient airs is in progress. There appears to be no differences in in vivo recovery using the two techniques.

PUBLICATIONS

1. Schiffer CA, Aisner J: Failure of post filtration leukopheresis plasma to increase granulocyte yields. Transfusion 17:500-503, 1977.

2. Schiffer CA: Principles of granulocyte transfusion therapy. Medical Clinics of North America 61:1119-1131, 1977.

3. Schiffer CA, Sanel FT, Young VB, Aisner J: Reversal of granulocyte adherence to nylon fibers using local anaesthetic agents: Possible application to filtration leukopheresis. Blood, 50:213-225, 1977.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06276-05 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
The Chemotherapy of Metastatic Testicular Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Stanley Ostrow, M.D.	Clinical Associate	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)
Urology Division, Department of Surgery, University of Maryland Hospital

LAB/BRANCH
Clinical Oncology Branch

SECTION
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INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dose reduction and delay in therapy has been necessary because of bone marrow suppression It is our assessment that this combination is active in testicular carcinoma at the dose and schedule given. However because of severe myelosuppression, initial dose reduction will have to be made i.e. 40 mg/m^2 adriamycin on day 1 and 60 mg/m^2 of cis-platinum on days 1 and 8 especially in patients receiving prior chemotherapy and radiotherapy.

PHS-6040
(Rev. 10-76)

Cis-platinum and adriamycin have been shown to have activity against testicular carcinoma. The purpose of the current BCRP Protocol #7606 is to test this drug combination in patients with genitourinary neoplasms including testicular carcinoma.

Patients have been given adriamycin, 60 mg/m^2 intravenously on Day 1 and cis-platinum 75 mg/m^2 intravenous infusion over 6 hours, days 1 and 8 every 2 weeks.

Prehydration with 1 liter normal saline and mannitol and lasix diuresis are also given. Doses are adjusted for BSP and white count and platelet count nadirs. Patients are to receive 6 courses of therapy. Seven patients with testicular carcinoma with metastases have been entered into study to date. There have been 3 complete and 2 partial responses. One patient with no measurable disease at the onset of therapy remains disease free twelve months after cessation of therapy. The one patient with no response had received multiple courses of combination chemotherapy prior to receiving platinum and adriamycin.

Toxicities were as follows: All 7 patients developed nausea and vomiting, myelosuppression (WBC $<$ 2000 cu mm) was seen in all patients, thrombocytopenia (platelet count $<$50,000 cu.mm) was seen in 6 patients. One patient developed reversible renal failure necessitating discontinuance of platinum for several courses.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06277-07 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Chemotherapy of Adult Acute Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul Chang, M.D.	Senior Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
Others:	Stephen Schimpff, M.D.	Head, Section of Infection Research	COB NCI
	Charles Schiffer, M.D.	Senior Investigator	COB NCI
	Joseph Aisner, M.D.	Senior Investigator	COB NCI
	Linda Edwards, R.N.		COB NCI
	Mary Scoltock, R.N.		COB NCI
	Viola Mae Young, Ph.D.	Head, Microbiology Section	COB NCI
	Prem Sarin, Ph.D.	Deputy Chief, Laboratory of Tumor Cell Biology	LTCB NCI

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A seven day continuous intravenous infusion of cytosine arabinoside in association with a three day schedule of intermittent daily doses of daunorubicin has been shown to be highly effective chemotherapy for the induction of remission in adults with acute non-lymphocytic leukemia. A complete remission rate of 60% is obtained with this combination. Addition of levamisole to this induction regimen does not enhance this response rate. Serum muramidase levels appear to accurately reflect disease activity in a large proportion of patients with acute non-lymphocytic leukemia. Carcinoembryonic antigen and serum immunoglobulin concentrations do not appear to accurately reflect disease activity. Similarly, biochemical testing of leukemic bone marrow cells does not allow for selection of favorable groups of patients to be treated with cytosine arabinoside (kinase-deaminase ratio) or daunorubicin alone (daunorubicin reductase level).

Reverse transcriptase has been identified in the bone marrow cells of leukemia patients prior to treatment and in morphologically normal peripheral cells during complete remission. One interpretation of this data is that morphologically and functionally normal granulocytes in the peripheral blood of leukemia patients in complete remission may be derived from leukemic cells.

I. Therapy:

A. Use of Biochemical Predictors of Disease Activity

Preliminary reports suggest that the ability to induce complete remission of acute non-lymphocytic leukemia with cytosine arabinoside and daunorubicin can be predicted by biochemical testing of bone marrow cells. Using the kinase-deaminase ratio to select patients to be treated with cytosine arabinoside and the daunorubicin reductase level to select patients to be treated with daunorubicin alone, we have attempted to give previously untreated patients with acute non-lymphocytic leukemia the drug which their biochemical tests predict they should be most sensitive to. Three patients with high ("favorable") kinase-deaminase ratios and low ("unfavorable") daunorubicin reductase concentrations received cytosine arabinoside alone without achieving complete remission. Twelve patients with higher daunorubicin reductase levels and low kinase-deaminase ratios received daunorubicin alone. Four patients achieved complete remission and one patient had a good partial remission. Sixty additional patients had low kinase-deaminase ratios and low daunorubicin reductase concentrations, or had technically unobtainable levels and were treated with other therapy (see B).

No relationship could be discerned between concentrations of kinase, deaminase, kinase-deaminase ratio, or daunorubicin reductase and attainment of complete remission, duration of complete remission, or survival from therapy in any of the treatment groups. This study did not confirm any usefulness of these enzyme assays in predicting attainment of complete remission with either cytosine arabinoside or daunorubicin alone or the combination of both drugs together.

B. Addition of Levamisole to Therapy of Acute Non-Lymphocytic Leukemia.

Half of the 60 patients mentioned above were treated with a three-day schedule of daunorubicin plus a seven-day infusion of cytosine arabinoside while the other 30 patients were treated with the same two drugs with the addition of levamisole given in a dose of 45 mg/m^2 orally twice a day for three days each week beginning with the second week of induction therapy and continuing until relapse or death. Both groups were similar with respect to median age and age distribution, distribution of sexes and number of patients with infection admitted to each arm. The remission induction rates were 63% without levamisole and 57% with levamisole. Median duration of remission and the median survival from the initiation of therapy were also similar for the two groups.

Analysis of other factors which may have affected achievement and duration of remission demonstrated that younger patients, patients who were uninfected at the time of admission and female patients had significantly higher remission induction rates than did their counterparts in the study. As yet there is no explanation why the female patients have done better in the study.

C. Maintenance Therapy for Remission in Adults with Acute Non-Lymphocytic Leukemia.

Following attainment of the first complete remission, patients were treated with high-dose methotrexate with citrovorum factor for three months, followed by intermittent courses of cytosine arabinoside and thioguanine given to hypoplasia every three months with patients in laminar air flow in protective isolation with oral nonabsorbable antibiotics. Of the 60 patients on this phase of the study, 6 have begun the late intensification phase with cytosine arabinoside and thioguanine. Only one of these patients has relapsed with 3 of these patients in their first complete remission more than two years since the initiation of treatment.

D. Future Plans

A new study is about to be undertaken using induction therapy consisting of three days of adriamycin with a 7-day infusion of cytosine arabinoside. Following attainment of remission, patients will be randomly assigned to receive either cytosine arabinoside and thioguanine given to hypoplasia intermittently, the same chemotherapy with the addition of neuraminadase-treated allogeneic myeloblasts, or the same chemotherapy plus splenectomy.

II. Biochemical Indicators of Disease Activity in Acute Non-Lymphocytic Leukemia:

A. Serum and Urine Muramidase Studies

We continue to confirm the utility of serum muramidase levels in differentiating between acute monocytic, myelomonocytic, and myelocytic leukemia. In addition, we continue to observe that serum muramidase levels accurately reflect disease activity in a large proportion of patients with acute non-lymphocytic leukemia. It has also become evident that patients with initially elevated serum muramidase levels who achieve complete remission and normal serum muramidase levels have a longer complete remission than those who achieve hematologic and clinical remission but maintain elevated serum muramidase levels.

B. Carcinoembryonic Antigen

We have observed that approximately 30% of adult acute non-lymphocytic leukemia or acute lymphocytic leukemia patients have abnormal levels of carcinoembryonic antigen in their serum. Unfortunately, these levels do not correlate well with disease activity. Most patients undergoing therapy have a rise for weeks or months in their plasma CEA whether or not they respond to therapy. In addition, when patients relapse the presence or absence of CEA in their plasma at that time does not correlate with findings at the time of presentation.

C. Serum Immunoglobulin Concentrations in Patients with Acute Non-Lymphocytic Leukemia

We have measured quantitatively serum IgA, IgD, IgE, and IgM in patients with acute non-lymphocytic leukemia prior to therapy, during therapy, during remission and relapse. Most patients with acute non-lymphocytic leukemia present with decreased levels of IgG in their serum. When complete remission is achieved these levels become normal.

D. Reverse Transcriptase in Leukemic Cells

This enzyme of presumed viral origin is being looked for in cells and plasma from our leukemia patients by Dr. Sol Spiegelman, New York. Results are preliminary at this point. Fifty patients have been studied prior to therapy and the enzyme was present in the white cells of each. In addition, most patients in complete remission continue to have the enzyme detectable in their white cells. We are now looking for the enzyme in the various fractions of the buffy coat of patients in remission and in autopsy specimens.

E. Cell-Mediated Immunity in Acute Non-Lymphocytic Leukemia

Cell-mediated immunity in acute non-lymphocytic leukemia, either at initial presentation or in subsequent relapse, was determined among 98 patients by recall-antigen skin testing and in 67 patients by attempted sensitization and challenge with dinitrochlorobenzene (DNCB). The leukemic patient's subsequent prognosis in terms of attainment of complete remission of his leukemia, duration of remission and survival could not be correlated with the pre-therapy demonstration of intact delayed cutaneous hypersensitivity.

F. Deoxynucleotide Terminal Transferase Studies

This DNA polymerase, originally found in normal thymic cells, has subsequently been identified in relatively high concentrations in the blast cells of most patients with childhood and adult acute lymphocytic leukemia. It has also been reported to be present in high concentrations in blasts from a minority of patients with chronic myelogenous leukemia in blast crisis, as well as a small fraction of patients with otherwise morphologically classified acute non-lymphocytic leukemia in whom histochemistry and Romanowsky staining are equivocal. Because of the suggestion that this enzyme may provide a means for classifying leukemic cells (and hence stratifying treatment for patients) in addition to the classical morphologic and clinical criteria, blast cells of all new patients with acute leukemia are being assayed for their terminal transferase activity in addition to more routine histologic studies.

Summary of Major Findings:

1. Daunorubicin and cytosine arabinoside given in intensive courses continue to produce a complete remission rate superior to that of other regimens tested at this institution or elsewhere.

2. Levamisole immunotherapy is of no benefit in the induction and maintenance therapy of adults with acute non-lymphocytic leukemia.

3. Serum muramidase levels are useful in following disease activity in adult acute non-lymphocytic leukemia.

4. Biochemical testing of leukemic bone marrow cells does not allow for selection of favorable groups of patients to be treated with cytosine arabinoside (kinase-deaminase ratio) or daunorubicin alone (daunorubicin reductase level).

5. Carcinoembryonic antigen is present in increased amounts in the minority of patients with acute non-lymphocytic leukemia. Serial determinations, however, are useless in following disease activity.

6. Cell-mediated immunity in leukemia patients prior to therapy may not be important in determining response.

7. The presence of reverse transcriptase in the white cells of leukemia patients in remission may indicate the necessity for further induction therapy. It may also suggest that "normal" granulocytes present during remission represent matured leukemic cells.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06280-06 COB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Combination Chemotherapy for Advanced Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Senior Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fifty patients with advanced Hodgkin's disease were treated with a combination of cyclophosphamide, vinblastine, procarbazine and prednisone (CVPP) in an every three week regimen. Patients who achieved complete remission were randomized to receive maintenance therapy consisting of monthly alternating CCNU and vinblastine or to followed on no therapy. Thirty-one patients (62%) achieved compelte remission with the fewest remissions being seen (3 of 8) in those patients who had previously received both chemotherapy and radiation therapy. Maintenance therapy to date has not significantly prolonged remission duration or survival. Patients who receive more than six courses of induction therapy have had longer remissions and fewer relapses than those receiving only six courses regardless of maintenance therapy status. CVPP is an effective regimen at inducing remissions in advanced Hodgkin's disease. Maintenance therapy has not yet been shown to be beneficial.

PHS-6040
(Rev. 10-76)

Project Description: Z01 CM 06280-06 COB

To determine if a combination of cyclophosphamide, vinblastine, procarbazine and prednisone (CVPP) is effective at inducing remissions in advanced Hodgkin's disease regardless of prior treatment status, and to assess the effects of maintenance therapy with CCNU and vinblastine on remission duraiton and survival.

Fifty-nine patients have been entered on study which was closed to further entries on December 31, 1975. Fifty patients are considered evaluable, 27 previously treated and 23 having received no prior therapy. Overall, 31 patients achieved a complete remission; 15/19 of those previously treated with irradiation only, 3/8 of those having previously received irradiation and chemotherapy and 13/23 of those without prior therapy have achieved remissions.

Myelosuppression was the major toxicity with leukopenia being seen more frequently than thrombocytopenia.

As of January 1, 1978, the 16 patients receiving maintenance therapy have not had significantly longer durations of remissions or durations of survival than the 14 patients not being maintained. There have been 11 relapses, five while maintenance therapy was being given.

Patients receiving greater than six courses of induction therapy have had longer remisison duration and fewer relapses regardless of whether maintenance therapy was given or not.

CVPP is an effective induction regimen for advanced Hodgkin's disease regardless of prior treatment status. Maintenance therapy has not yet shown any significant prolongations of remission or survival. This project is now closed.

Publications:

1. Diggs CH, Wiernik PH, Levi JA, and Kvols LK: Cyclophosphamide, vinblastine, procarbazine and prednisone with CCNU and vinblastine maintenance for advanced Hodgkin's disease. Cancer 39:1949-1954, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06293-07 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
A Teaching and Consultation Service in Medical Oncology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Br.	COB NCI
Other:	Robert J. Esterhay, M.D.	Senior Investigator	COB NCI
	Paul Chang, M.D.	Senior Investigator	COB NCI
	Joseph Aisner, M.D.	Senior Investigator	COB NCI
	Charles Schiffer, M.D.	Senior Investigator	COB NCI
	Charles Diggs, M.D.	Senior Investigator	COB NCI
	David VanEcho, M.D.	Senior Investigator	COB NCI
	Karen Lichtenfeld, M.D.	Senior Investigator	COB NCI

COOPERATING UNITS (if any)
University of Maryland School of Medicine, Johns Hopkins University School of Medicine, Greater Baltimore Medical Center, the University of Missouri at Kansas City School of Medicine, Good Samaritan Hospital, Baltimore, VA Hospital, Baltimore.

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The dissemination of information concerning the latest techniques in the diagnosis and therapy of cancer patients is of paramount importance in advancing the attack on this disease. By training potential physicians early during their student and house officer years, such information will be brought to the community in a more orderly and useful way than is sometimes possible. By seeing patients in consultation, we have the opportunity to educate the patient's physician as to modern techniques of management for a given neoplastic disease. These teaching functions that we engage in are important in elevating the level of care available to patients with cancer in this area.

PHS-6040
(Rev. 10-76)

Project Description: Z01 CM 06293-07 COB

The objectives are to teach the theory and practice of medical oncology to medical students and house officers; to acquaint such individuals with newer aspects of chemotherapy, radiotherapy, and surgery as they apply to the management of neoplastic diseases; and to provide a consultative service for difficult patients with neoplastic diseases for other physicians in the Baltimore area.

During their second year, medical students from the University of Maryland are assigned to this service on a regular basis for their physical diagnosis class. Students from the University of Maryland and Johns Hopkins are assigned here during elective periods for a clinical clerkship in medical oncology. In addition, house officers from the Greater Baltimore Medical Center and the University of Missouri at Kansas City School of Medicine have been assigned here for elective periods of their house officer year. University of Maryland Assistant Residents in Medicine rotate through the Clinical Oncology Branch on a regular basis. Second year medical residents from the Good Samaritan Hospital (a University of Maryland affiliated hospital) rotate through the Branch on a monthly basis. Physicians in the community refer patients here for out-patient consultation. If the patient meets the qualifications for one of our protocol studies, he is accepted in transfer and admitted to BCRP. If not, the patient is completely evaluated as an out-patient and returned to his physician with specific recommendations for therapy.

Students and house officers who have rotated through the BCRP are recognizing that medical oncology is a bona fide subspecialty of internal medicine. They have learned that patients with neoplastic diseases require special care based on special knowledge. It has been suggested by some local private physicians that the general care of patients with neoplastic diseases in the community has advanced because of our consultation service.

The dissemination of information concerning the latest techniques in the diagnosis and therapy of cancer patients is of paramount importance in advancing the attack on this disease. By training potential physicians at this time, early during their student and house officer years, such information will be brought to the community in a more orderly and useful way than is sometimes possible. By seeing patients in consultation, we have the opportunity to educate the patient's physician as to modern techniques of management for a given neoplastic disease. Both of these teaching functions we engage in are of significant import in elevating the level of care available to patients with cancer in this area.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06295-06 COB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Census: A Remote Access Patient Information Storage and Retrieval System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William L. Robinson	Computer Specialist	COB NCI
	Joann Gustafson	Systems Analyst	COB NCI
	Robert J. Esterhay, M.D.	Senior Investigator	COB NCI

COOPERATING UNITS (if any)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The CENSUS system is a set of computer programs which enables the storage and retrieval of a data base of information on cancer patients treated at the Baltimore Cancer Research Program. The computer programs and the data base are accessed from remote terminals connected to a commercial time-sharing computer facility. Structured data input programs facilitate the addition of new information to the data base. A flexible retrieval program produces patient listings sorted by patient name, cancer diagnosis, attending physician, status, and/or research protocols. A medical secretary updates the data base daily and then retrieves a CENSUS report which lists all patients currently on the Clinical Oncology inpatient service and Clinical Oncology clinic visits made that day. A general purpose information management software library performs data base manipulations and retrievals which previously required the development of special purpose computer programs. Several programs have been written to produce administrative and accounting tabulations of the data stored on the system. The system has been in operation since August of 1971, and the data base currently contains records for over 1700 cancer patients.

PHS-6040
(Rev. 10-76)

OBJECTIVES:

1. To compile, update, and retrieve from a data base of medically relevant information on cancer patients that is centralized, readily accessible and maintained by a single non-technical individual.

2. To generate a daily report on all inpatients and their locations, along with their vital statistics and current therapy data to be distributed to all involved in the tasks of clinical research, patient care and administration.

3. To develop flexible data retrieval programs which generate a variety of useful patient summaries keyed on any of several patient parameters.

METHODS EMPLOYED:

1. Information is compiled and recorded daily in a data input log by a medical secretary.

2. A computer program accepts the input datum from the secretary, checks for a logical content and accuracy, and records it on the data base.

3. A second computer program generates a Daily Census Report which reflects the updated status of the data base.

4. The report is then distributed to the scientific and professional staff, remote contracting laboratories and administrative office.

5. The programs and data base are executed and stored on a commercial time-sharing service.

6. Mass memory disc files and remote terminals from the storage and access mediums.

The Daily Census Report has been utilized by all sections and services of the Cancer Center.

1. Admissions Officer: used to assign new patients to their physicians and to distribute the patient load evenly among the physicians.

2. Clinical Associates: used at evening sign-out rounds as a checklist of patients with special problems that may require extra care overnight.

3. Clinical Oncology Branch: the master retrieval programs are most useful to the physician performing a chart review to evaluate treatment protocols and who requires listings of the patient population which are sorted by status, cancer diagnosis, research protocol, etc.

4. Clinical Research Pharmacy Service: used to fill the unit drug cart patient trays.

5. Sections of Microbiology and Infection Research: provides the laboratory staff and outside contracting laboratories with relevant information on patients whose cultures are being analyzed. The permanent records of all beds a patient has occupied provide a useful epidemological tool in detecting the spread of infectious organisms. The data base is also accessed by computerized microbiology reporting systems at the BCRP (see Project Report Z01 CM 06039-05 BCR P).

6. A general purpose information management software library now performs data base manipulations and retrievals which previously required the development of special purpose computer programs.

7. Several programs have been written to produce administrative and accounting tabulations of the data stored on the system.

The content and flexibility of the retrieval summaries generated by the Census System have provided the Research Center with a valuable "index" to patient records. The daily routine of update and audit have eliminated the inaccuracies and incompleteness of previous disjoint and ill-kept data bases. Six years of continuous operation of the Census System have provided BCRP with a substantial data base of information on cancer patients. Future developments will concentrate on effective utilization of the information thus obtained. These developments will be in the form of programs to correlate census data with data compiled by other data systems at the BCR P.

A systems analyst has been added to the project professional staff to provide, in part, codification and standardization of research protocols, and to provide the data processing support necessary for maintaining a comprehensive data-base management system involving treatment data which is based upon existing CENSUS applications.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06299-05 COB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Therapy of Patients with Metastatic Sarcoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul Chang, M.D.	Senior Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
Others:	Nicholas Bachur, M.D., Ph.D.	Chief, Laboratory of Clinical Biochemistry	LCB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Oncology Branch

SECTION

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NCI, NIH Baltimore, Maryland 21201

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The combination of adriamycin and actinomycin-D has been used to treat patients with inoperable or metastatic sarcoma. Although the combination increases the percentage of objective improvements in these patients, there is also an increased incidence of adriamycin cardiac toxicity which may suggest that the total cumulative adriamycin dose may need to be reduced further in future patients on this combination. Previously-treated patients are being entered onto a broad Phase II trial of high-dose cis-platinumdiamminedichloride and some responses have been seen.

Cerebral metastases have been identified either clinically or at autopsy in approximately 10% of patients with advanced sarcomas who have been treated with chemotherapy. Such metastases were especially common in patients with malignant fibrous histiocytoma and rhabdomyosarcoma. Patients who develop brain metastases have a somewhat longer median survival than patients who do not develop brain metastases.

PHS-6040
(Rev. 10-76)

A. Therapy of Previously Untreated Patients with Advanced Sarcomas

Adriamycin has now become the cornerstone of therapy of inoperable and metastatic sarcomas, having been identified as the most effective single drug in this group of tumors. It has, however, significant dose-limiting toxicity, in that 10-30% of patients who receive more than 550 mg/m^2 (cumulative dose) later suffered irreversible cardiac failure.

Actinomycin-D, an antineoplastic antibiotic with a similar mechanism of action, is known to be an effective palliative drug in approximately 20-30% of patients with advanced sarcomas. It is thus believed to be the second most active single agent for chemotherapy of these tumors. A pilot study combining adriamycin 60 mg/m^2 IV q 3 weeks with actinomycin-D 300 mcg/m^2 IV qd days 8 through 12 has therefore been undertaken to attempt to improve the remission rate and ultimately the survival of patients with metastatic or inoperable sarcomas. The rationale for this schedule was based on our previous demonstration that drug induced hepatic dysfunction could influence the metabolism and therapeutic efficacy of adriamycin. It has also been hypothesized that because of the similarity of mechanism of action of the two agents, giving them simultaneously could be counterproductive.

Of the 19 evaluable patients there have been 2 complete responses, 3 partial remissions, 7 improvements and 7 patients have had no response. Duration of remission ranges from 1+ to 15+ months. The most significant side effect has been the development of congestive heart failure, probably due to adriamycin cardiac toxicity, in 4 patients who received 500 mg/m^2 cumulative dose of adriamycin. Two of these patients died of their cardiac toxicity, and 2 continue to respond to therapy for this complication. Other toxicities have included nausea with vomiting, mucositis, leukopenia, thrombocytopenia and anemia. Of interest is the observation that mucositis and myelosuppression appear to be moderately increased in frequency compared with the expected toxicity from either drug given alone; however, mucositis and myelosuppression were not seen concomitantly in any patient. Pharmacokinetic studies are being performed to try to elucidate this observation.

The clinical results with this combination appear at least as good as the therapeutic results using adriamycin alone. There is a definite increase in the number of patients with objective improvement and a moderate increase in acute toxicity. The increased incidence of cardiac toxicity with this regimen, however, suggests that the total cumulative dose of adriamycin may need to be reduced further in future patients.

New patients are currently being accrued into two studies, one an examination of the effects of hyperthermia with adriamycin on advanced sarcomas, and the other an attempt to enhance the therapeutic index of adriamycin by combining it with the polyene antibiotic amphotericin-B. Each of these projects is reported separately.

B. Therapy of Previously Treated Patients

Patients who have received prior therapy including adriamycin have been entered into Phase II drug studies. We have previously reported the inefficacy of IV methyl-CCNU and azotomycin in such studies. Two of 9 such previously treated patients with advanced sarcomas have had impressive partial responses to cis-platinumdiamminedichloride given in varying dosages and schedules. The major toxicities have been nausea, vomiting, renal dysfunction and occasional myelosuppression. Because of this, patients are currently being entered into a broad Phase II trial of cis-platinum given as a six-hour infusion with hydration and diuresis at a dose of 100 mg/m^2 every three weeks. The results of this recently initiated study are reported separately.

C. CNS Metastases in Patients with Sarcomas

Cerebral metastases from patients with sarcomas are extremely infrequent. Recently, however, it has been noted that such metastases may be increasing in frequency, perhaps as the result of successful therapy and prolongation of life through the use of agents such as adriamycin which does not penetrate the blood brain barrier. In an attempt to confirm these results, the records of 114 patients with inoperable or metastatic sarcoma have been examined. In 11 of our patients cerebral metastases have been identified, six on the basis of clinical findings and diagnostic procedures and five on the basis of these plus post-mortem documentation. Two of the 11 had brain metastases at the time systemic metastatic disease was first recognized. No additional cases of brain metastases were found in asymptomatic patients who were autopsied. There appears to be a somewhat longer median survival for patients who eventually develop brain metastases than for patients who do not develop brain metastases. The median survival of patients after the diagnosis of brain metastases, however, is very brief. These results may be somewhat skewed by the significantly higher incidence of CNS metastases in patients with malignant fibrous histiocytoma (3/11 patients) and rhabdomyosarcoma (4/15 patients).

Publications:

1. Chang P, Levine MA, Wiernik PH, and Walker MD: A phase II study of intravenously administered methyl-CCNU in the treatment of advanced sarcomas. Cancer 37:615-619, 1976.

2. Chang P, Wiernik PH: Combination chemotherapy with adriamycin and streptozotocin. I. Clinical results in patients with advanced sarcomas. Clin Pharmacol Therapeu 20:605-610, 1976.

3. Chang P, Riggs CE Jr, Scheerer MT, Wiernik PH, and Bachur NR: Combination chemotherapy with adriamycin and streptozotocin. II. Clinical pharmacologic correlation of augmented adriamycin toxicity caused by streptozotocin. Clin Pharmacol Therap 20:611-616, 1976.

4. Chang P: Management of soft tissue sarcomas: Current status. Amer J Med Sci 273(3):244-258, 1977.

5. Chang P: Progress in the treatment of osteosarcoma. Med Clin NA 61:1027-1039, 1977.

6. Chang P and Wiernik PH: Phase II study of azotomycin (NSC-56654) in sarcomas. Cancer Treat Rep 61:1719-1720, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM 06913-05 COB

PERIOD COVERED
October 1, 1977 through Sept. 30, 1978

TITLE OF PROJECT (80 characters or less)

Bronchial Brushing for Pulmonary Infiltrates in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner, M.D.	Senior Investigator	COB NCI
Others:	Stephen C. Schimpff, M.D.	Head, Section of Infection Research	COB NCI
	Viola M. Young, Ph.D.	Head, Section of Microbiology Research	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

Pathology and Radiology Departments, University of Maryland Hospital

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since the myelosuppressed patient with cancer frequently develops pneumonias which are difficult to diagnose, aggressive diagnostic techniques are warranted. This study established a step-wise, orderly method of diagnosing unexplained pulmonary infiltrates utilizing transtracheal bronchial brushing, fibroptic bronchoscopy, or percutaneous lung biopsies when other methods have failed. Fifty-seven patients with diffuse or nodular infiltrates have undergone such procedures. Fourteen patients were not clinically infected; 8 proved to have pulmonary metastases; 5 proved to have interstitial fibrosis; and one proved to have a collapsed lobe. Forty-three patients were clinically presumed infected. Twenty-eight were proven to have pulmonary infection. Twenty-four of the 28 were etiologically documented by these procedures. Fifteen of the 43 patients had no etiology established by any method, although 12 of the 15 patients were receiving broad spectrum antibiotics at the time of the procedure. The diagnoses included a variety of microorganisms: bacterial, protozoal, and fungal. Significant but non-fatal complications occurred and were acceptable risks for the patient population. There was no mortality associated with the procedures, and despite pancytopenia, bleeding was a problem in only 3 patients.

Project Description: Z01 CM 06913-05 COB

The immunologically compromised and myelosuppressed cancer patient frequently develops pulmonary complications, especially infections. Such pneumonias are a contributing factor in the demise of many of these patients. A major reason for the high mortality lies in difficulty of establishing an etiological agent and thereby providing appropriate antimicrobial therapy. Since there is an established low yield of noninvasive culture techniques for pneumonia when occurring in such patients, more aggressive diagnostic techniques are warranted. Myelosuppressed patients frequently carry gram negative organisms and fungi in their naso-oropharynx which will contaminate expectorated sputum. Furthermore, such patients rarely produce sputum spontaneously. The purpose of this study is to establish a method for diagnosing unexplained pulmonary infiltrates when conventional methods such as sputum induction and transtracheal aspiration have been unsuccessful. This study utilizes a transtracheal approach with selective bronchial brushing, peroral fibroptic bronchoscopy, and percutaneous lung biopsies when other methods have failed.

As of March, 1978, 57 patients with either diffuse or nodular pulmonary infiltrates have undergone transtracheal bronchial brushing followed by other procedures. Conventional methods including transtracheal aspiration failed to establish a diagnosis in each case. Fourteen patients were not considered clinically infected. Eight of them proved to have pulmonary metastases of which only one was diagnosed by this technique. Five patients for whom no diagnosis was obtained by brushing subsequently proved to have interstitial fibrosis by open-lung biopsy or necropsy. One additional patient had a collapsed lobe at thoracotomy. Forty-three patients were clinically presumed infected. Ultimately, 28 of these 43 patients were proven to have pulmonary infection, and 24 of the 28 were etiologically documented by brushing. For 15 of the 43 presumed infected patients, no etiology could be established by any method. Twelve of these 15 patients were receiving broad spectrum antibiotics at the time of the procedure. Diagnoses established by the brushing procedure included pneumonias involving such organisms as Aspergillus, Mucorales, Torulopsis glabrata, Staphylococcus aureus, Mycobacterium tuberculosis, Pneumocystis carinii, as well as aerobic and anaerobic Streptococci. In addition, one case of metastatic sarcoma was identified by cytology. Significant but nonfatal complications included hemoptysis, pneumothorax, and cervical cellulitis occurring in a total of nine patients. There was no mortality associated with this procedure. Despite pancytopenia, bleeding was a significant problem in only 3 patients.

Proposed Course:

Continue Investigation.

Publications:

Articles:

1. Aisner, J., Schimpff, S.C., Bennett, J.E., Young, V.M., Wiernik, P.H.: Aspergillus infections in patients with cancer: In association with fire-proofing materials in a new hospital. JAMA 235:411-412, 1976.

2. Aisner, J., Kvols, L.K., Sickles, E.A.,Schimpff, S.C., Wiernik, P.H.: Transtracheal selective bronchial brushing for pulmonary infiltrates in patients with cancer. Chest 69:367-371, 1976.

3. Aisner, J., Schimpff, S.C., Sutherland, J.C., Young, V.M., Wiernik, P.H.: Torulopsis glabrata infections in patients with cancer: Increasing incidence in relationship to colonization. Am J of Med 61:23-28, 1976.

4. Aisner, J., Schimpff, S.C., Wiernik, P.H.: Treatment of invasive Aspergillosis: Relation of early diagnosis and treatment to response. Ann Int Med 86:539-543, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06915-05 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Staging Laparotomy and Combined Modality Therapy in Early Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	Charles H. Diggs, M.D.	Investigator	COB-NCI
Other:	Robert Slawson, M.D.	Associate Professor	UMH-Balto

COOPERATING UNITS (if any)
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University of Maryland Hospital, Baltimore, Maryland

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NCI, NIH Baltimore, Maryland

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1	1	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aim of this study was to determine the value of staging laparotomy in Stage I and II Hodgkin's Disease. It was also to evaluate different modalities of therapy for pathologically documented Stage I and II Hodgkin's disease. Following completion of initial clinical evaluation, patients were randomized to receive laparotomy or no laparotomy if the clinical evaluation showed no evidence of Hodgkin's disease beyond Stage II. Those patients receiving no laparotomy received extended field irradiation alone and those who received laparotomy were randomized to receive limited field irradiation alone, extended field irradiation alone, or limited field irradiation plus MOPP chemotherapy. Fifty patients have been randomized to date. All patients have achieved complete remission and there have been relapses in each treatment group with the exception of the group that received adjuvant chemotherapy. The numbers of patients studied in each group are too small to make any firm evaluation of this study at this time possible. Although there is a suggestion that patients who receive adjuvant chemotherapy may do better, confirming our previous work.

PHS-6040
(Rev. 10-76)

1. To determine whether modification of radiation therapy ports on the basis of staging laparotomy findings will significantly improve survival of patients with clinically localized Hodgkin's disease as compared to radiation therapy administered without staging laparotomy.

2. To determine whether the addition of adjuvant chemotherapy to limited field irradiation therapy in patients with pathologically documented localized disease is beneficial.

Patients with newly diagnosed Hodgkin's disease undergo complete clinical evaluation including x-ray, hematologic, biochemical and nuclear medicine studies. If none of these studies suggests more advanced disease than Stage II, patients are randomized to receive laparotomy or no laparotomy. If no laparotomy is performed, patients receive extended field irradiation therapy. If laparotomy is performed, patients are randomized to receive either limited field irradiation therapy, extended field irradiation therapy, or limited field irradiation therapy followed by chemotherapy using MOPP.

Fifty patients have been entered onto study at this point. All patients were able to achieve a complete remission and there have been relapses in each group except those treated with adjuvant chemotherapy. Approximately half of the relapses have occurred in patients with "E" stage disease of the lung in patients who received only irradiation therapy. Because of the limited number of patients in each treatment group, it is too early to make any firm conclusions from this study.

Publications:

Levi, J.A., and Wiernik, P.H.: Unfavorable prognosis and therapeutic implications of limited extranodal Hodgkin's disease. Amer J Med, 63: 365-372, 1977.

Levi, J.A., O'Connell, M.J., and Wiernik, P.H.: Patterns of relapse in Stage I, II, and III-A Hodgkin's disease: Influence of initial therapy and implications for the future. Int J Rad Oncol, 2: 853-862, 1977.

Levi, J.A., Wiernik, P.H.: Therapeutic implications of splenic involvement in stage IIIA Hodgkin's disease. Cancer 39: 1949-1954, 1977.

Wiernik, P.H.: Hodgkin's Disease, In Price's Textbook of Medicine, Haynes & Row, Hagerstown, Md., (in press), 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06916-05 COB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Pilot Protocol for Treatment of Patients with Stage III and IV Diffuse Histiocytic Lymphoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Senior Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was designed to determine the feasibility of administering high dose intensive chemotherapy for patients with advanced diffuse histiocytic lymphomas using measures to prevent infection. Patients with diffuse histiocytic lymphoma who were found to be stage III or IV after appropriate staging techniques were placed in reverse isolation in a laminar air flow unit and begun on oral non-absorbable antibiotics. They then received high dose chemotherapy with cyclophosphamide 1500 mg/m^2 IV day one, adriamycin 60 mg/m^2 IV day one, vincristine 1.4 mg/m^2 IV days one and eight and a ten day course of oral prednisone at 40 mg/m^2. Eight patients were treated. Myelosuppression was the most frequently noted toxicity and though often severe was brief. Results show four complete responses, one partial response and three failures. There have been three deaths of those patients achieving complete response, however. One patient died from adriamycin cardiotoxicity, one from disseminated fungemia and one patient with renal failure. The patient who has achieved a partial remission achieved a complete remission on further therapy. Although the results of this study indicate that high dose chemotherapy with these agents is effective at achieving complete responses, the toxicities of this approach appear prohibitive.

PHS-6040
(Rev. 10-76)

Project Description:

This project was designed to determine whether high dose intensive combination chemotherapy of patients with diffuse histiocytic lymphomas utilizing measures to prevent infection will result in improved remission rates and prolonged survivals.

Patients with biopsy proven diffuse histiocytic lymphoma who were shown to be stage III or stage IV after staging procedures were placed in reverse isolation in a laminar air flow unit and given prophylactic non-absorbable oral antibiotics. These patients then received therapy with cyclophosphamide 1500 mg/m^2 IV day one, adriamycin 60 mg/m^2 IV day one, vincristine 1.4 mg/m^2 IV days one and eight and a ten day course of oral prednisone 40 mg/m^2. Because cyclophosphamide and adriamycin are both myelosuppressive when given individually and since these agents are both used in full dosage here, measures to prevent infection are utilized.

Eight patients were entered onto this study. Reverse isolation and the administration of oral antibiotics was well tolerated. Myelosuppression was severe though brief. However, one patient did die from disseminated fungal infection. There were four complete responses noted, although three of these patients have died, one from the aforementioned fungal infection, one from adriamycin cardiotoxicity and the other from renal failure. One patient has achieved a partial response and there have been three failures. Thus, 50% of patients so far treated have achieved complete remissions at the expense of drug toxicity. Infection seems to be the major limiting factor of this study.

Since disseminated diffuse histiocytic lymphoma is the most malignant variety of the non-Hodgkin's lymphomas, more aggressive chemotherapeutic regimens are needed for its treatment. Infectious complications on this regimen have caused it to be discontinued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06917-05 COB

PERIOD COVERED
October 1, 1977 through Sept. 30, 1978

TITLE OF PROJECT (80 characters or less)

CVP versus CAVP for Disseminated Non-Hodgkin's Lymphomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Senior Investigator	COB NCI
Others:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Seventy-two previously untreated patients with Stage III or IV non-Hodgkin's lymphoma were randomized to receive either CVP (C = 1.5 gm/m^2 IV day 1, V = 1.4 mg/m^2 IV days 1 and 8, P = 40 mg/m^2 PO days 1-10) or CAVP (C = 1.0 gm/m^2 IV day 1, A = 45 mg/m^2 IV day 1, V = 1.4 mg/m^2 IV days 1 and 8, P = 40 mg/m^2 PO days 1-10). Fifty-two patients have completed therapy. All patients achieving complete remission then received CCNU 100 mg/m^2 PO day 1, bleomycin 20 mg/m^2 IM days 1 and 8, and prednisone 40 mg/m^2 PO days 1-10 (CVP) every three weeks for three courses. 11/23 (48%) CVP patients achieved CR (6/11 nodular, 5/12 diffuse) as did 18 out of 29 CAVP (11/14 nodular, 7/15 diffuse). Myelosuppression was moderate with both regimens as well as with CBP treatment. Median remission durations from the end of each induction therapy will be in excess of 21 months (range 4 to 42+) for CVP and in excess of 17 months for CAVP (range 2 to 53+). Two out of 11 CVP patients and 3 out of 18 CAVP patients have relapsed to date. No patient entering CR has died of progressive lymphoma. CVP and CAVP both appear to be effective regimens for remission induction in patients with advanced non-Hodgkin's lymphomas. The addition of adriamycin may be advantageous for nodular lymphomas.

Patients who are found to have advanced non-Hodgkin's lymphomas, either Stage III or IV on the basis of extensive clinical and pathologic staging procedures are randomly assigned to receive therapy with either cyclophosphamide 1500 mg/m^2 IV on day 1, vincristine 1.4 mg/m^2 on days 1 and 8, and prednisone 40 mg/m^2 orally on days 1-10, or cyclophosphamide 1000 mg/m^2 IV on day 1, adriamycin 45 mg/m^2 IV on day 1, vincristine 1.4 mg/m^2 days 1 and 8 intravenously, and prednisone 40 mg/m^2 orally on days 1-10. Patients who achieve pathologically documented complete remissions then undergo consolidation therapy with CCNU, bleomycin and prednisone. A second randomization is then carried out with patients randomly assigned to receive no further therapy or to receive intermittent high dose Cytoxan monthly intravenously.

Seventy-two patients have undergone randomization to date with 35 patients receiving CVP therapy and 37 patients receiving CAVP therapy. Twenty patients are still receiving treatment. Eleven of 23 or 48% of the CVP patients have achieved complete remission, as have 15 of 23 patients receiving CAVP. The median duration of remission for the complete responders on CVP will be in excess of 21 months, and in excess of 17 months for CAVP. There have been two relapses in the CVP group and 3 after therapy with CAVP. No differences in the two therapy arms in remission induction or toxicities have been observed. It is too early to evaluate the effect of maintenance therapy.

Conclusions so far to be drawn from this project are: (1) the combination of Cytoxan, vincristine and prednisone is effective in inducing remission in patients with disseminated non-Hodgkin's lymphomas; (2) the addition of adriamycin to CVP may be advantageous for nodular lymphomas.

Publications:

1. Diggs, C.H., and Wiernik, P.H.: Cyclophosphamide, vincristine and prednisone (CVP) vs. cyclophosphamide, adriamycin, vincristine and prednisone (CAVP) for disseminated non-Hodgkin's lymphomas. Proc. Amer. Soc. Clin. Onc., p. 288, 1977.

2. Diggs, C.H. and Wiernik, P.H.: The non-Hodgkin lymphomas. In Practice of Medicine Harper & Row (publ.), (In press.)

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PERIOD COVERED
October 1, 1977 through Sept. 30, 1978

TITLE OF PROJECT (80 characters or less)

Streptozotocin, Bleomycin, CCNU & Adriamycin Treatment of Advanced Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Senior Investigator	COB NCI
Others:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Seventeen patients with advanced previously treated Hodgkin's disease received therapy with a combination of streptozotocin 500 mg/m^2/day IV days 1-5, CCNU 100 mg/m^2 orally day 1, adriamycin 45 mg/m^2 IV day 1, and bleomycin 15 mg/m^2 IM days 1 and 8 at 28 day intervals. The overall response rate was 59% with 6 patients (35%) achieving complete remissions and 4 patients (24%) entering partial remissions. Median duration of complete remission is 21+ months with a range of 4+ to 41+ months. The median duration of survival from the start of therapy for the complete responders was 26+ months with a range of 7+ to 47+ months. Myelosuppression occurred regularly and was severe in 25% of courses. Other toxicities included bleomycin pulmonary toxicity in three patients, renal tubular dysfunction secondary to streptozotocin in 3 patients and hepatic dysfunction in 3 patients. SCAB has been shown to be an active combination of agents which is not cross-resistant to MOPP-type regimens.

To determine clinical usefulness of new combination chemotherapy for advanced Hodgkin's disease in patients no longer responsive to conventional chemotherapies.

Patients with advanced biopsy-proven Hodgkin's disease who are no longer responsive to other agents receive streptozotocin 0.5 gm/m^2 daily for 5 days intravenously, CCNU 100 mg/m^2 PO day 1, bleomycin 15 mg/m^2 IM days 1 and 8, and adriamycin 45 mg/m^2 IV day 1. Cycles are repeated each 28 days.

Seventeen patients have been treated with this regimen. Six patients (or 35%) have achieved a complete remission, and an additional 4 patients (or 24%) have entered partial remissions, for an overall response rate of 59%. This combination of agents appears to have a definite activity in this previously-treated group of patients with Hodgkin's disease.

MOPP chemotherapy for advanced Hodgkin's disease has become considered as standard for these patients. Between 60 and 80% of patients treated with MOPP will achieve complete remission, yet there remains a sizable percentage of these patients who do not achieve complete remission. Therefore, the development of effective alternative regimens is necessary either for achieving complete remissions in greater percentages of patients or for treating patients who have not responded to MOPP. Streptozotocin, CCNU, adriamycin and bleomycin all have definite activity against Hodgkin's disease, and since each has different toxicities the combination of the four drugs would seem feasible. These agents as used in this combination have shown activity in patients with previously-treated Hodgkin's disease, although myelosuppression was perhaps more of a problem than with MOPP. A contributing factor, however, would be that all these patients had received prior chemotherapy, making their marrows more sensitive to chemotherapeutic agents. Because of the activity of this regimen, it is now being tried in previously untreated patients with advanced Hodgkin's disease.

Publications:

1. Levi, J.A., Wiernik, P.H., and Diggs, C.H.: Combination chemotherapy of advanced previously-treated Hodgkin's disease with streptozotocin, CCNU, adriamycin and bleomycin. Medical and Pediatric Oncology 3, pp. 33-40, 1977.

2. Diggs, C.A., Wiernik, P.H. and Aisner, J.A.: SCAB (Streptozotocin, CCNU, Adriamycin, Bleomycin) for advanced untreated Hodgkin's disease. (Abs.) Proc. Am. Soc. Clin. Oncol. 19:370, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06919-04 COB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Initial Drug Therapy for Metastatic Renal Cell Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Hahn	Senior Investigator	COB NCI
Other:	S.C. Schimpff	Head, Section of Infection Research	COB NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

None

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NCI, NIH Baltimore, Maryland 21201

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0.2	0.2	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Metastatic renal cell carcinoma has proven to be generally unresponsive to all agents evaluated to date including most alkalating agents, antimetabolites, and antibiotics. Progesterone derivatives or androgens give a low number of partial remissions and about a 20% subjective response rate. Many currently available chemotherapeutic agents have not yet been adequately evaluated including three agents for which there are preliminary data to suggest some responsiveness, i.e., CCNU, vinblastine and ThioTEPA. These three drugs were therefore tested in a prospective randomized trial of therapeutic efficacy in patients with metastatic renal cell carcinoma. Patients whose tumor progressed on this study were then treated with bleomycin for which there were also some early data suggesting possible value in this disease. A partial response was seen in one of seven ThioTEPA treated patients, zero of nine CCNU treated patients and zero of ten vinblastine treated patients although two of the latter had an objective response. None of seven bleomycin treated patients had an objective response.

Objectives;

Renal carcinoma is a solid tumor of notorious resistance to chemotherapy and radiation. Surgery may be curative in some cases, but the overwhelming percentage of cases proceed to metastatic disease and death within two years of diagnosis. Most previous chemotherapy studies have been inadequate. Several previous well-controlled studies indicated that adriamycin had no activity and provera had only minimal activity. Drugs that have shown some efficacy in preliminary trials included CCNU with a 10 to 15% response rate, vinblastine with approximately the same response rate and bleomycin which had less of a response rate. In addition, a drug derivative of ThioTEPA which had been tried in the Soviet Union, was very promising with approximately 50% response rate. Therefore, a study was set up to randomize between vinblastine, CCNU and ThioTEPA for the first line of therapy and if the patient relapsed from this therapy then a second round of therapy would be bleomycin unless there was sufficient reason not to give the bleomycin because of chronic obstructive pulmonary disease.

Major Findings:

Twenty-six patients with metastatic hypernephroma were randomized among the three first line forms of therapy. Three groups were equivalent in age, prior therapy, and time from diagnosis to therapy and incidence of CNS metastases. Nine patients received CCNU therapy and 7 of these were evaluable. This therapy resulted in no partial responses, 2 stabilizations and 5 progressions. Vinblastine has been received by 10 patients. There have been 2 objective responses, 1 stabilization and 7 progressions. ThioTEPA has been received by 7 patients. There have been 1 short-lived partial response, 1 stabilization and 5 progressions. Seven patients have received bleomycin and 2 have had stabilization, but one has had progressive disease. Myelosuppression was the major side effect in each of the initial drug therapies with no complications other than therapy delayed resulting from this. Emesis occurred in the CCNU group (9 patients). Survival time was equivalent in all 3 groups and did not differ from historical controls. Brain metastasis was associated with early death in 3 out of 4 patients and a lack of response to therapy in all patients. Thus, minimal activity was demonstrated for ThioTEPA and vinblastine and no responses were seen in CCNU or bleomycin.

It was thought possible that part of the failure of response was the inability to deliver the drug to the site of tumor in adequate concentrations. Therefore, ThioTEPA (chosen because of its minimal but present response in the current study) was given in combination with dimethylsulfoxide (DMSO) in hopes of augmenting the possible effectiveness of thioTEPA. This study is documented elsewhere (Z01 CM 6976 01 COB).

Publications:

Hahn, D.M., Schimpff, S.C., Ruckdeschel, J.C., and Wiernik, P.H.: Single-agent therapy for renal cell carcinoma: CCNU, vinblastine, ThioTEPA, or bleomycin. Cancer Treat. Rep. 61: 1585-1587, 1977.

Aisner, J. and Wiernik, P.H.: ThioTEPA and dimethylsulfoxide in the therapy of renal cell carcinoma. Cancer Treat. Trials (in press), 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06921-05 COB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Adriamycin Therapy for Advanced Mycosis Fungoides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	Charles H. Diggs, M.D.	Senior Investigator	COB NCI

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study has been designed to determine the efficacy of adriamycin therapy for the treatment of advanced mycosis fungoides. Adriamycin has been administered to patients with biopsy proven diagnosis of mycosis fungoides who were refractory to any prior therapy. Disease status was advanced in all patients with extensive skin involvement and lymphadenopathy. Visceral disease was frequent. Adriamycin was administered in a dose of 60 mg/m^2 IV every three weeks and a minimum of three courses was given beyond complete response. Maintenance therapy consisted of methotrexate 15 mg/m^2 IM twice weekly and Cytoxan 750 mg/m^2 IV every three weeks. Thirteen patients were entered onto this study. There have been three complete responses, five partial responses and three improvements, with two failures. Toxicity has been mild and it would appear that adriamycin is an active agent for the treatment of advanced mycosis fungoides.

This project was designed to determine the efficacy of adriamycin therapy for the treatment of advanced mycosis fungoides.

Adriamycin was given to patients with biopsy proven diagnosis of mycosis fungoides and who had received previous therapy but now were refractory. Lymphadenopathy was present in all cases and all patients had extensive skin disease. Adriamycin was administered in a dose of 60 mg/m^2 IV every three weeks with dosage modification for hepatic dysfunction or if significant myelosuppression occurred. A minimum of three courses were given and if a complete response occurred, a further three courses beyond that point were given. Maintenance therapy was administered with methotrexate 15 mg/m^2 IM twice weekly and Cytoxan 750 mg/m^2 IV every three weeks. Of the 13 patients entered onto study, there have been three complete responses (23%), five partial responses (39%), three improvements (23%) and two failures. Toxicity was mild with only a moderate degree of myelosuppression noted. It would appear, therefore, that adriamycin is an effective agent for the treatment of advanced mycosis fungoides.

Once mycosis fungoides reaches an advanced stage there is often rapid progression to eventual death. If single agents can be shown to be effective in this disease, perhaps more effective combinations of agents can be used to prolong survival time. Adriamycin would appear to be one of these agents. This project will now be closed.

Publications:

1. Levi JA, Diggs CH, and Wiernik PH: Adriamycin therapy for advanced mycosis fungoides. Cancer 391967-1970, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06939-03 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Methotrexate, Oncovin, L'Asparaginase and Dexamethasone Therapy for Adults with Acute Lymphocytic Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert J. Esterhay, Jr., M.D.	Senior Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to improve the remission induction rate of acute lymphocytic leukemia in adult patients utilizing vincristine and dexamethasone with intermittent moderate doses of methotrexate followed by L'asparaginase. A second objective is to decrease the incidence of severe infections during induction therapy by utilizing oral non-absorbable antibiotics. A third objective is to determine whether intermittent high dose methotrexate with leucovorin rescue and with vincristine and dexamethasone will prolong remission duration. The fourth objective is to determine the efficacy of moderate and high dose methotrexate in preventing meningeal leukemia and to correlate the clinical effectiveness with the cerebrospinal fluid methotrexate levels. To date 25 patients have been entered onto this study. Fourteen patients had no prior treatment, eleven had prior treatment. For the no prior treatment patients, six patients are still on study and eight are off study. For the prior treatment patients, five patients are still on study and six patients are off study. For the no prior treatment patients, complete remissions have been achieved in nine of fourteen patients that went through induction. One patient is too early to evaluate. For the prior treatment patients, eight patients achieved complete remission.

The purpose of this project is to improve the remission induction rate of Acute Lymphocytic Leukemia (ALL) in adult patients utilizing vincristine and dexamethasone with intermittent moderate doses of methotrexate followed by L'asparaginase. A second objective is to decrease the incidence of severe infections during induction therapy by utilizing oral non-absorbable antibiotics. The third objective is to determine whether intermittent high dose methotrexate with leucovorin rescue and with vincristine and dexamethasone will prolong remission duration in a significant percentage of adult patients with ALL. The fourth objective is to determine the efficacy of moderate and high dose methotrexate in preventing meningeal leukemia and to correlate clinical effectiveness with the cerebrospinal fluid methotrexate levels.

Patients 15 years of age and over with a diagnosis of ALL will be eligible. Induction therapy will consist of methotrexate 100 mg/m^2 IV push on day one only, dexamethasone 6 mg/m^2 orally daily, vincristine 2 mg (regardless of body surface area) IV push on day two only, and L'asparaginase 500 IU/kg IV infusion over a 30 minute period on day two only. The induction therapy is to be continuous with the second course beginning on day eleven, the third course on day 21, etc. Patients achieving a complete remission precede to consolidation therapy which consists of continuing with only methotrexate and L'asparaginase. The consolidation therapy consists of six cyclic courses which begins the day after the completion of induction therapy. Patients remaining in complete remission after six courses of consolidation therapy will proceed to cytoreduction therapy, which consists of vincristine, dexamethasone, high dose methotrexate (100 mg/kg) followed by citrovorum factor (5% of the high dose methotrexate dose (mg/mg) over three days). Cytoreduction therapy is given monthly for a total of 12 courses. Maintenance therapy begins after the completion of cytoreduction and is continued indefinitely until bone marrow relapse with each course given at four week intervals. Maintenance therapy consists of vincristine on day one, dexamethasone orally on days one through five, methotrexate (15 mg/m^2) orally weekly, and 6-mercaptopurine orally daily.

A total of 25 patients have been entered onto this study. Fourteen patients had no prior treatment, 11 had prior treatment. For the no prior treatment patients, one is too early to evaluate, and 13 have been through induction. Complete remissions have been achieved in nine of 13 patients that went through induction. One patient that died during induction from infection had a definite clinical improvement by bone marrow exam and at autopsy had no evidence of lymphoblasts. For the prior treatment patients, eight have achieved complete remission and four have died. The major toxicity with the program is related to the E. coli strain of L'asparaginase. Almost every patient has had to be switched to the Erwinia strain. There is a 69% complete remission rate for the previously untreated patients and a 73% complete remission rate for the previously treated patients.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06942-03 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Development of the Acoustic Microscope as a Useful Tool in Hematology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB-NCI
Other:	C. F. Quate, Ph.D.	Professor of Applied Physics & Electrical Engineering	Stanford U. Stanford, Ca.

COOPERATING UNITS (if any)
Stanford University, Stanford, California

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The acoustic microscope, which utilizes microwave sound rather than light or electron beams to reproduce an image, is investigated in this study for its possible usefulness in hematologic research. Early results indicate that appropriate resolution is possible.

PHS-6040
(Rev. 10-76)

Project Description: Z01 CM 06942-03 COB

The acoustic microscope, developed at Stanford University, utilizes microwave sound rather than light or electrons to reproduce the image of a microscopic object. Objects to be examined by the instrument need no fixing or staining. We are endeavoring to adapt this instrument for the examination of peripheral blood and bone marrow by addressing ourselves to problems of resolution. early results are encouraging and indicate that this instrument may ultimately be useful for rapid examination of hematologic specimens. The best method of preparing the specimen for examination in under current investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06944-03 COB

PERIOD COVERED
October 1, 1977 through Sept. 30, 1978

TITLE OF PROJECT (80 characters or less)

Treatment of Oat Cell Carcinoma of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner, M.D.	Senior Investigator	COB NCI
Others:	Robert J. Esterhay, Jr., M.D.	Senior Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Oat cell carcinoma of the lung is a rapidly fatal disease and aggressive chemotherapy is warranted. These studies evaluated combination chemotherapy with active agents (cyclophosphamide, adriamycin, and VP 16-213, as the basic regimen and CCNU, methotrexate, vincristine and procarbazine as an alternating regimen). Immunotherapy with MER was shown in a previous study not to be useful for response rate, duration or survival. Thirty-nine evaluable patients were entered onto the first study with the base combination, and the median duration of survival on this study will be greater than one year. Previously irradiated patients did less well than previously untreated patients, and the complete plus partial remission rate overall was 78%. For previously untreated patients, the complete plus partial response rate was 90%. Nine of the patients on that study continue on therapy beyond one year, and two patients are beyond two years and off all drugs for 8 and 5 months respectively. The subsequent study seeks to improve the complete remission rate and duration of response and survival by utilizing an alternating non-cross-resistant combination therapy program. Thirty-seven patients have been entered onto the second study, and preliminary evaluation shows no difference in the response rate. It is too early to develop stable survival data. Toxicity in these studies was manageable by dose modification.

PHS-6040
(Rev. 10-76)

Since nearly all patients with small cell carcinoma of the lung (oat cell) die of metastases, this disease is considered disseminated at the time of presentation. Regardless of the form of localized therapy, patients have a short survival, and aggressive combination chemotherapy is warranted to treat the systemic disease. The combination of cyclophosphamide, adriamycin and VP 16-213 is very active in this disease, and the duration of survival for all patients treated with this combination chemotherapy program alone will be in excess of one year. Therefore, the results from this study with chemotherapy are at least as good as any reported study to date. Immunotherapy as used in the first study did not apparently add significantly to the response rate or survival. Therefore, in an attempt to improve upon complete response rate and duration of response and survival, another approach utilizing a second combination chemotherapy program, non-cross-resistant to the first, was tested. The current study therefore randomizes between cyclophosphamide, adriamycin and VP 16-213 given continuously to relapse, followed by second-line therapy with the alternating combination vs. cyclophosphamide, adriamycin, VP 16-213 given until complete remission or stable response, and then beginning an alternating program of the first and second combination agents. Patients are stratified according to whether they have limited or disseminated disease. In addition, the previous study showed a central nervous system recurrence rate of approximately 30%. Therefore, to test the role of potential prophylaxis on the central nervous system, all patients who achieve complete remission on this protocol are randomized to receive or not receive 3,000 rads of cranial whole-brain irradiation as prophylaxis for CNS recurrence.

As of April 1978, 39 evaluable patients completed the first study. Three patients presented with non-measurable disease and are all surviving free of recurrent disease at greater than one year. Of 36 evaluable patients, 50% achieved complete remission, and 28% achieved a partial remission. Patients that had received prior irradiation did significantly worse than the patients who had no prior therapy. Thirty-one evaluable patients had no prior therapy. Complete response rate was 58% and partial response rate 32%. Duration of response and survival was significantly greater in the non-treated group, and the duration of response and survival was greater for patients with limited as compared to widespread disease. The duration of survival in the first study is projected to be in excess of one year, and therefore the results are at least as good as any reported study today utilizing combined modality therapy. Nine of the patients on the study continue on therapy beyond one year from diagnosis and two patients are beyond two years off all drugs for 8 and 5 months respectively. The second protocol testing the concept of alternating combination chemotherapy programs has accrued 37 patients, stratified according to extent of disease and randomized between the two treatment programs. At the present time, there are no apparent differences in response rate and duration of survival, although it is too early to develop stable survival data. Fourteen patients have been randomized to receive or not receive whole-brain irradiation, and it is too early to conclude whether this has had any significant effect. Toxicities in both studies have been moderately severe and included alopecia, nausea and vomiting, and blood count suppression. Toxicities were generally manageable by dosage modification. Sixteen patients had serious infectious episodes during periods of neutropenia and five such patients died from these infections. There were no other drug-related deaths, and most patients received all therapy as outpatients. MER toxicity included fever, chills, local pain and weeping ulcer formation. MER-induced fever during periods of neutropenia confused the management of the neutropenic patients and resulted in multiple hospitalizations.

Proposed Course:

Continue investigation.

Publications:

1. Aisner, J., Wiernik, P.H., Esterhay, R.J. jr: Treatment of small cell carcinoma of the lung with cyclophosphamide, adriamycin and VP 16-213 with or without MER. in Adjuvant Therapy of Cancer, Salmon, S.E. and Jones, S.E. (Eds), Elsevier/North Holland Press, 1977, pp. 245-250.

2. Esterhay, R.J. jr: Current concepts in the management of small cell carcinoma of the lung. Am J Med Sci 274:232-246, 1978.

3. Aisner, J., Satterfield, John R., jr., Aisner, S.C., Pyeatte, J.C., Wiernik, P.H.: Inflammatory changes related to active tuberculosis: confusion with oat-cell carcinoma of the lung on cytology specimens. Chest 73:670-671, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06947-02 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Amphotericin-B and Adriamycin Combination Therapy of Refractory Neoplasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul Chang, M.D.	Senior Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
Others:	Nicholas Bachur, M.D., Ph.D.	Chief, Laboratory of Clinical Biochemistry	LCB NCI

COOPERATING UNITS (if any)

None

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro and in vivo laboratory studies indicate that amphotericin enhances the cell uptake and cytotoxicity of many compounds, including some of the antitumor antibiotics. This study is an attempt to determine if there is a clinically recognizable enhancement of the therapeutic index of adriamycin when combined with amphotericin.

Amphotericin-B, a polyene, antifungal, antibiotic, is able to augment the cellular uptake of many compounds, including certain antitumor antibiotics. Other laboratories have demonstrated an enhanced therapeutic index both in vitro and in vivo for BCNU with amphotericin. Other experiments with tumor cells resistant to actinomycin-D because of decreased cellular uptake of the drug, demonstrate an augmented cellular uptake and enhanced cytotoxicity of actinomycin-D when these drug resistant cells were exposed to amphotericin with actinomycin-D. Studies in our own laboratory with L1210 leukemia have demonstrated impressive enhancement of survival of mice receiving both amphotericin and the anthracycline antibiotic, daunorubicin, compared with leukemic mice receiving either drug alone.

Recent clinical results have been reported with a combination of adriamycin, BCNU, cyclophosphamide and amphotericin. Responses were obtained in patients with acute non-lymphocytic leukemia, carcinoma of the breast and myeloma already resistant to the combination of adriamycin, BCNU and cyclophosphamide alone. Although fever and chills accompanied the administration of amphotericin, myelosuppression was not felt to be uniformly increased.

This study will use amphotericin combined with adriamycin in patients who are already resistant to adriamycin, to see if there is any augmentation of the therapeutic index of adriamycin. Only 4 patients have been treated thus far with no reportable results. Adriamycin pharmacokinetics are also being determined in these patients.

Publications:

1. Klein, M.E. and Frayer, K: Alterations in secondary adriamycin (A) resistance by amphotericin (AT) and hyperthermia. Proceedings of the American Society of Clinical Oncology 19:84, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06948-02 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Treatment of Chronic Myelocytic Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Esterhay, M.D.	Senior Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)
None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The first study for the treatment of chronic myelocytic leukemia is designed to determine whether or not more intensive treatment directed against the presumed abnormal myeloblasts can postpone or prevent myeloblastic transformation. The second study is designed to assess the effectiveness of anthracycline antineoplastic therapy in the induction treatment of the transformation phase of chronic myelocytic leukemia once it does develop. The objective of the first study is to determine whether CCNU and Ara-C begun at the time of diagnosis of CML without busulfan can postpone or prevent myeloblastic transformation while the objectives of the second study are to assess the effectiveness of the addition of daunorubicin to hydroxyurea, 6-mercaptopurine and prednisone as induction treatment of the blastic or "resistant" phase of CML. In the first study, patients are randomized to control treatment with busulfan followed by continuous "maintenance" busulfan. CCNU and Ara-C given every six weeks starting at the time of entry into the study. In the second study patients are randomly assigned to one of two treatment programs designed to compare the relative effectiveness of daunorubicin, hydroxyurea, 6-mercaptopurine and prednisone with or without the addition of MER immunotherapy. As of June, 1977, 16 patients have been entered onto these two studies. Eleven patients on the first one and five on the second.

None of the traditional methods of treatment have significantly increased the survival of patients with chronic myelocytic leukemia (CML). The median duration of survival of patients with CML is still approximately 3-3.5 years, about the same as that described in 1924 for both treated and untreated patients. Myeloblastic transformation is the main cause of death; approximately 70% of patients die in this stage of the disease. Since there is no striking effective treatment for this phase of the disease once developed, a reasonable approach to prolong survival is to prevent the onset of the blast crisis. The first study is designed to determine whether or not more intensive treatment directed against the presumed abnormal myeloblast can postpone or prevent myeloblastic transformation. The second study is designed to assess the effectiveness of daunorubicin in the induction treatment of the blastic phase of CML once it does develop.

The objective of the first study is designed to determine whether CCNU and Ara-C begun at the time of diagnosis of CML without busulfan can postpone or prevent myeloblastic transformation. The objectives of the second study are as follows: to assess the effectiveness of the addition of daunorubicin to hydroxyurea, 6-mercaptopurine, and prednisone as induction treatment of the blastic or "resistant" phase of CML; to evaluate whether intermittent intravenous cyclophosphamide, added to maintenance therapy with hydroxyurea, 6-mercaptopurine and prednisone can prolong the duration of remission when compared to historical controls; to determine whether early immunotherapy with MER will increase the probability of achieving complete remission and prolonging its duration.

In the first study, patients with CML are randomized to "control" treatment consisting of induction of remission with busulfan followed by continuous "maintenance" busulfan to maintain the WBC between 4,000 and 15,000/mm^3 or to CCNU and Ara-C every 6 weeks starting at the time of entry into the study. In the second study patients with the blastic or "resistant" phase of CML are randomly assigned to one of two treatment programs designed to compare the relative effectiveness of daunorubicin, hydroxyurea, 6-mercaptopurine and prednisone with or without the addition of MER immunotherapy to prior experience with the hydroxyurea, 6-mercaptopurine, prednisone combination. All patients with "resistant" CML who achieve complete or partial remission status after induction treatment will receive the same maintenance treatment with daily hydroxyurea, 6-mercaptopurine, pulsed prednisone and daunorubicin to the maximum allowable cumulative dose.

As of June, 1978, 16 patients have been entered onto these two studies. Eleven patients were entered onto the first study to postpone or prevent myeloblastic transformation. Three of the 11 patients are off study, having developed the blastic or "resistant" phase of CML. These three patients plus an additional two patients who presented with the blastic phase of CML were entered onto the second study. No responses, have been obtained on the second study.All five patients are off study and have subsequently died.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06949-02 COB

PERIOD COVERED
October 1, 1977 through Sept. 30, 1978

TITLE OF PROJECT (80 characters or less)

Combination Chemotherapy for Metastatic Carcinoma of the Colon

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Senior Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

None

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TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Twenty-one patients with advanced metastatic adenocarcinoma of the colon or rectum were treated with a combination of intravenous Methyl CCNU and Ftorafur, an analogue of 5-fluorouracil. In addition, 13 patients were randomized to receive MER. Two previously untreated patients, neither of whom had received MER, achieved partial remissions lasting 52 and 31 weeks. Neither patient had hepatic involvement with disease. Four patients had stabilization of disease lasting up to 14 weeks. Three of these patients had received MER. Hematologic toxicity of this regimen was severe with 65% of all patients treated having platelet counts below 100,000/mm^3 at some time during the course of therapy. 33% of patients had white blood cell counts of 2,000/mm^3 at some time during therapy. Although others have reported optimistic results from similar drug combinations, the results of this study are not encouraging. It is postulated that hepatic conversion of ftorafur to 5-FU may occur less well in patients with hepatic dysfunction. Further studies of ftorafur metabolism in humans are needed.

1. To assess the value of the combination of intravenous Methyl CCNU and ftorafur in patients with metastatic adenocarcinoma of the colon or rectum.

2. To assess the value of immunotherapy in addition to combination chemotherapy.

Adenocarcinoma of the large bowel is a disease which has been consistently resistant to all forms of nonsurgical therapy. Although 5-fluorouracil remains the most commonly used medication for this disease, combinations of chemotherapeutic agents have been shown to be superior to 5-FU as a single agent. The combination of Methyl CCNU and 5-FU has given high response rates, and this study was designed to assess whether the intravenous form of Methyl CCNU combined with ftorafur with or without immunotherapy was beneficial.

Twenty-one patients were treated on this regimen, all of whom had objectively measurable sites of metastatic disease. There were 16 males and 5 females with a median age of 56 years. Eighteen of these patients were fully ambulatory at the start of therapy. Only one patient had received prior chemotherapy and 4 patients had received local radiation therapy. Thirteen patients had hepatic involvement with metastatic disease either alone or in combination with other sites of metastasis. Of the 21 patients treated, only two patients (or 9%) were able to achieve partial remissions of their disease. Neither of these patients had hepatic involvement with metastatic disease. One patient had a non-surgically-resectable abdominal mass, the other patient having a left neck mass. Severe hematologic toxicity was noted with this regimen, with 65% of all patients having platelet counts below 100,000/mm^3 at some time during their therapy.

Possible reasons for the lack of a high percentage of responders may be that intravenous Methyl CCNU is not as active a drug as oral Methyl CCNU. The hematologic toxicity with thrombocytopenia being severe would seem to indicate, however, that the drug was active. Ftorafur, on the other hand, must be metabolized to 5-fluorouracil for it to become active. Since animal studies have suggested that hepatic tissue is a site in which ftorafur is metabolized to 5-FU, it is possible that patients with metastatic disease in their livers do not metabolize ftorafur to its active form. Immunotherapy with MER, likewise, did not seem to increase the response rate.

Because of the lack of efficacy of this treatment regimen, this project is closed to further patient accrual.

Publications:

1. Diggs, C.H., Wiernik, P.H., Smythe, A.C., and Sutherland, J.C.: Intravenous Methyl-CCNU and ftorafur MER for metastatic colorectal carcinoma. Proc Amer Assoc for Cancer Res, p 134, May, 1977.

2. Diggs, C.H., Wiernik, P.H., and Smythe, A.C.: IV methyl CCNU and ftorafur with or without methanol-extracted residue of BCG for metastatic adenocarcinoma of the colon. Cancer Treat. Rep. 61:1581-1583, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06952-02 COB

PERIOD COVERED
October 1, 1977 through Sept. 30, 1978

TITLE OF PROJECT (80 characters or less)

Mycosis Fungoides Cooperative Study Group

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Senior Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Ocology Branch	COB NCI
Others:	Stanford I. Lamberg, M.D.	Chairman, Steering Committee, Baltimore City Hospital, Baltimore, MD	

COOPERATING UNITS (if any)

All institutions in the Cooperative Study Group.

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Clinical Oncology Branch

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NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS: 0.75	PROFESSIONAL: 0.75	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Mycosis Fungoides Cooperative Group was founded in order to study both the natural history of and various treatment regimens for mycosis fungoides in all stages of the disease. To date, 400 cases of pathologically documented mycosis fungoides have been accrued and follow-up information is current on more than 95% of these patients. All data have been computerized and are in the process of being examined to seek correlations with various demographic, history and physical examination, laboratory and pathologic data. Forty patients to date have been randomized on the chemotherapy arm which attempts to study both chemotherapy, radiotherapy and topical therapy for the disease. Of these 40 patients, 4 have been entered from the Baltimore Cancer Research Center onto treatment arms. One patient failed therapy, one patient died from bleomycin pulmonary toxicity, and two patients are presently being followed. for response. Of the 400 study patients who are being followed but not on chemotherapy protocols from the study group, there have been 14 from the BCRC. All patients continue to be followed by the BCRC. There are no firm conclusions yet from this study.

Mycosis fungoides is frequently an indolent skin disease that presents many years before any diagnostic pattern appears on pathologic specimens. It is the intent of this study group to assess what the course of mycosis fungoides is from the time of pathologic documentation as well as what the course of pre-mycosis fungoides lesions may be. It is also the intent to study chemotherapy in patients with advanced cutaneous disease and in patients with visceral mycosis fungoides.

Patients who have received previous therapy for mycosis fungoides are presently being followed on no protocol therapy by the Cooperative Group in order to determine both the natural history of the disease and to accumulate more information concerning these patients. Patients who have received no prior therapy are being randomized to one of several treatment regimens. Patients with poor prognosis cutaneous disease without visceral mycosis fungoides, that is, those patients who have plaque disease over greater than 10% of their body surface area and palpable lymphadenopathy, or those patient with multiple tumors with or without lymphadenopathy, are being randomized to receive electron beams, radiotherapy or topical nitrogen mustard therapy. In addition, some patients are being randomized to receive no adjuvant systemic therapy, single-dose methotrexate therapy at 500 mg/m^2 followed by citrovorum rescue every 3 weeks, with dosage increments on each successive course, or to low-dose CHOP chemotherapy with cyclophosphamide 500 mg/m^2 IV, adriamycin 40 mg/m^2 IV, and vincristine 1.4 mg/m^2 IV plus prednisone 60 mg/m^2 orally days 1-5 repeated every 21 days. Those patients with visceral mycosis fungoides are allocated to one of three treatment regimens. Regimen 1 consists of methotrexate 500 mg/m^2 with dosage increments as described above, with citrovorum rescue alone. Regimens 2 and 3 consist of methotrexate with citrovorum rescue, adriamycin, bleomycin and azarabine, either given concurrently or given on successive weeks.

Four patients have been randomized from the BCRC to receive chemotherapy. One patient failed low-dose CHOP, one patient experienced fatal bleomycin pulmonary toxicity, the other two patients are still being treated. There are an additional 11 patients who are being followed on therapy at the BCRC, having been entered only in the demographic part of this study.

Four hundred cases have been accrued for follow-up study, and 40 patients have been randomized to receive one of the therapy arms. These patients are now being analyzed by the headquarters office and the results should be available soon. Patients will still be accrued on study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM-06953-02 COB

PERIOD COVERED

October 1, 1977 through Sept. 30, 1978

TITLE OF PROJECT (80 characters or less)

Chemotherapy for Metastatic Carcinoma of the Breast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner, M.D.	Senior Investigator	COB NCI
Others:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

Cancer and Leukemia Group B

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Combination chemotherapy for metastatic carcinoma of the breast utilizing combinations of active single agents can produce high response rates with improvement in the patients' survival. There are many active combinations with response rates which vary from 40-80%. There is a wide variation in reported response rates, but there are few prospective comparative trials of the various treatment regimens, and few studies which stratify for the prognostic variables. The current study randomly compares 3 active combination chemotherapy regimens for response rates, duration, and survival. In addition, the current study evaluates the effect of MER immunotherapy upon response rate, duration, survival, or its effect on drug toxicities. Since carcinoma of the breast is a very heterogenous disease and there are multiple treatment arms in this protocol, a large cooperative group effort is necessary to stratify for all the variables and the treatment arms. The protocol was activated October 11, 1976, and 219 patients have been entered onto protocol. Preliminary evaluations of this study reveal no differences in any of the six treatment arms. Stable survival data are not available at the present time.

Since the introduction of the 5-drug combination chemotherapy program for advanced breast cancer, there have been a large number of studies utilizing permutations of the drugs and their scheduling. In addition, the anthracycline antibiotic, adriamycin, has been used in several regimens in an attempt to improve response rate, duration or survival. All of these studies have shown that combination chemotherapy can improve the response rate, duration and survival for patients with metastatic disease as compared to treatment with single agents. Few of such studies, however, prospectively compare the various treatment regimens that have been utilized. Metastatic carcinoma of the breast is a heterogenous disease with many factors influencing the course of the metastatic disease. Thus, in order to do a prospective comparative trial of several different chemotherapy programs, a large group effort is necessary to stratify for these prognostic factors. The current study randomly compares 3 combination chemotherapy regimens, each of which has been shown to be an effective treatment program for metastatic breast cancer. In addition, the current study evaluates the role of immunotherapy utilizing MER intradermal injections for its potential to increase response rate, duration, survival, or alleviate drug toxicity. The role of MER immunotherapy is also being evaluated in a prospectively randomized manner. All treatment programs are clearly stratified for the recognized prognostic variables.

As of April 1978, 219 patients have been entered onto protocol from multiple institutions. The 219 patients have been roughly equally distributed among the various treatment groups. There are 199 evaluable patients. However, all interval analyses of data are encoded to avoid recognition and avoid investigator bias. At the present time, there are no statistical differences among any of the encoded arms.

Proposed Course:

Continue Investigation.

Publications:

1. Morris D, Aisner J, Wiernik PH: Horizontal pigmented banding of the nails in association with adriamycin chemotherapy. Cancer Treat Rep 61:499-501, 1977.

2. Morris D, Aisner J, Elias EG, Wiernik PH: Mastectomy as an adjunct to combination chemotherapy. Arch Surg 118:282-284, 1978.

3. Aisner J: Management of disseminated carcinoma of the breast. Am J Med Sci 275:4-16, 1978.

4. Aisner J and Wiernik PH: (Chemotherapy for recurrent or inoperable carcinoma of the breast) Nowotwory (Neoplasms - Polish) 28:80-89, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06954-02 COB

PERIOD COVERED
October 1, 1977 through Sept. 30, 1978

TITLE OF PROJECT (80 characters or less)

Chemotherapy for Carcinoma of the Head and Neck

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	E. George Elias, M.D., Ph.D.	Professor of Surgery, University of Maryland Hospital	
Others:	Joseph Aisner, M.D.	Senior Investigator	COB NCI

COOPERATING UNITS (if any)

Department of Surgery, University of Maryland Hospital

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Advanced squamous cell carcinoma of the head and neck or esophagus have very poor five-year survivals despite aggressive local treatment with surgery, radiotherapy or both. Many such patients die of metastatic disease. Therefore, a pilot trial of aggressive combination chemotherapy is warranted to determine if an improved response rate or survival can be obtained. Bleomycin, cis-platinum II with hydration, and high-dose methotrexate with citrovorum rescue are agents with activity in these tumors. The current study utilizes a combination of these 3 agents to determine toxicity, response rate and duration. Twenty-three patients with stage III and IV were treated with a single course of combination chemotherapy. Fourteen of 23 patients showed a greater than 50% response. Nine of these patients underwent extirpative surgery and tolerated it well. Toxicities have been manageable by careful hydration and careful folinic acid reversal. There were two drug-related deaths.

PHS-6040
(Rev. 10-76)

Advanced squamous cell carcinoma of the head and neck have a very poor survival rate despite extensive surgery or aggressive radiotherapy, and many of these patients develop inanition. Some of the patients develop metastases despite control of the local tumor. Several single agents have been identified with activity in these tumors and combination chemotherapy may thus be warranted. The current pilot study uses a combination of three active agents: bleomycin, cis-diamminodichloroplatinum with hydration, and methotrexate with folinic acid reversal, to test the toxicity of such a combination program and to determine if such combination therapy might increase response rate or duration or make the tumors more technically resectable.

As of April 1978, 23 patients with stage III and IV (M-0) squamous cell carcinoma of the head and neck were treated with a single course of combination chemotherapy. Ten patients had received prior treatment with surgery or radiotherapy while 13 patients were previously untreated. Fourteen of 23 patients showed greater than a 50% response. Nine of these 13 patients underwent extirpative surgery and tolerated it well. Three of the previously untreated patients had complete responses at the primary site. Toxicity from the chemotherapy included severe nausea and vomiting, diarrhea, renal toxicity, skin toxicity, alopecia, and bone marrow suppression. There were two drug-related deaths, one from nephrotoxicity and one from methotrexate bone marrow suppression. In general, toxicities were manageable by careful hydration and careful folinic acid reversal.

Proposed Course:

Continue investigation.

Publications:

1. Elias EG, Chretien PB, Monnard E, Wiernik PH: Combination chemotherapy in advanced squamous cell carcinomas of head and neck. Proc ASCO/AACR 19:376, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06955-02 COB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Adjuvant Chemotherapy and Combination Chemoimmunotherapy in the Treatment of Advanced Malignant Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karen Lichtenfeld, M.D.	Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

Department of Surgery, University of Maryland

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NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	1

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

There are only small numbers of patients in all arms of the study but the results are similar to those obtained in larger groups. In the patients receiving DTIC plus MER there is approximately a 29% partial response rate with responses seen mainly in patients with disease confined to the skin. In the group receiving DTIC alone there is a 25% partial response rate again involving intracuteanous metastases. There is still no evidence that adjuvant chemotherapy delays the time to progressive disease.

PROJECT DESCRIPTION: Z01 CM 06955 02 COB

There have been 29 evaluable patients entered on study. In the adjuvant arm of the protocol, six patients received DTIC alone and eight patients have been randomized to receive no therapy. The median survival from the date of randomization is 343+ days in the no treatment group and 288 in the DTIC group. This is not statistically significant. In the group of patients with advanced and measurable disease eight patients received DTIC alone and the median duration survival is 185 days. Seven patients recevived both DTIC and MER and the median survival is 80 days. There is no statistical difference the two groups. There are two patients receiving DTIC alone who are too early to evaluate.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06956-02 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Measurement of 5-Hydroxyindoleacetic Acid and Beta-2 Microglobulin in Cerebrospinal Fluid of Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karen Lichtenfeld, M.D.	Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
Others:	Thomas Koch, M.D.		

COOPERATING UNITS (if any)

Department of Clinical Pathology, University of Maryland Hospital, Baltimore, Md.

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1	0.5	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

A method has been developed for accurate determination of 5-Hydroxyindoleacetic acid and 5-Hydroxytyyptamine. Because of the considerable time involved in developing and perfecting this method there is no clinical data as yet. The methodology is currently the subject of a paper which will be submitted for publication shortly.

Sixty-two cerebral spinal fluids have been analyzed for beta-2-microglobulins. Althought the data is not as yet completed it appears that beta-2-microglobulin is elevated in the cerebropsinal fluids of patients with both leukemia and lymphoma who have documented central nervous system disease by other criteria, such as presence of blasts on cytocentrifuge and protein elevations. Because most of the lumbar punctures were obtained because of clinical suspicion of central nervous system disease, the elevation of the beta-2-microglobulin tended to be confirmatory rather than predictive. Beta-2-microglobulins also appeared to decrease with remission induction and do not appear to be affected by intrathecal chemotherapy. Therefore, although it appears that beta-2-microglobulins are increased in patients with CNS involvement, clinical criteria are probably better predictors of disease.

This study was undertaken in order to evaluate whether cerebrospinal fluid levels of beta-2 microglobulins or 5-hydroxyindoleacetic acid could serve as markers or predictors for CNS malignancy. It has been shown that 5-hydroxyindoleacetic acid decreases with spinal cord injury. Beta-2 microglobulins have not been studied in the CNS of patients with various disease states.

In evaluating the methods of determination of 5-hydroxyindoleacetic acid, it was found that the various methods were associated with inadequate specificity, sensitivity or simplicity. Therefore, we at first set out to develop a separation method which would permit accurate quantitation of serotonin and 5-hydroxyindoleacetic acid.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06957-02 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Nitrous Oxide in the Treatment of Refractory Adult Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karen Lichtenfeld, M.D.	Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
Others:	Martin Helrich		

COOPERATING UNITS (if any)

Department of Anesthesiology, University of Maryland Hospital, Baltimore, Md.

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Three patients have been entered into this study thus far and one of these patients was treated with nitrous oxide on two separate occasions. Therefore we have evaluated four clinical situations. The first patient had a maximum period of inhalation of 2½ hours which she tolerated extremely well with no unusual toxicity. The second patient was given an inhalation of 6 hours which she tolerated equally well. The patient who was studied twice tolerated a 40% concentration of nitrous oxide for periods up to 12 hours with only occasional interruption of nitrous oxide inhalation for periods of 1 to 5 minutes. The interruptions were caused by some nausea and vomiting which was eventually well controlled by hydroxyzine. During all trials there was some nausea and mild emesis during study. No patient experienced changes in vital signs or apparent renal, hepatic, or metabolic toxicity. Arterial blood gases remained within normal limits throughout the study for all patients. There were no effects on perpherial white counts or bone marrow examinations in any of the patients. We plan to use higher concentrations of nitrous oxide and longer exposure times in future subjects.

PHS-6040
(Rev. 10-76)

Project Description: Z01 CM 06957-02 COB

It has been documented that prolonged inhalation of nitrous oxide over a period of four or more days can lead to bone marrow aplasia. Although there is some clinical data suggesting that high peripheral counts and perhaps bone marrow can be suppressed in leukemia patients, there have been no systematic clinical trials. This protocol is a phase I evaluation of nitrous oxide in the treatment of adults who have failed conventional anti-leukemic agents. The objectives of the study are to determine the efficacy of nitrous oxide in suppressing myelopoiesis and to establish guidelines for the administration and dosage of this therapeutic modality.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06959-02COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Effect of General Anesthetic Agents on Platelet Aggregation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karen Lichtenfeld, M.D.	Investigator	COB NCI
	Charles A. Schiffer, M.D.	Senior Investigator	COB NCI
	Martin Helrich		

COOPERATING UNITS (if any)

Department of Anesthesiology, University of Maryland Hospital, Baltimore, Maryland 21201

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

This work has been completed and has been submitted to Anesthesiology for consideration for publication. The abstract of the paper is as follows:

Because of evidence suggesting that volatile anesthetics interfere with platelet aggregation *in vitro*, we investigated platelet function in patients exposed to general anesthesia during brief surgical procedures. In twelve patients undergoing elective operations, blood was sampled before anesthesia, after induction but prior to surgery, during surgery, and post-operatively. Platelet aggregation by ADP, ephinephrine, and collagen was similar during each sampling period in all but two subjects. During brief exposure to general anesthetics, there appears to be no clinically important inhibition of platelet function.

PHS-6040
(Rev. 10-76)

Project Description:

Although there is in vitro data suggesting that general anesthetic agents have an inhibitory effect on platelet aggregation, this has never been substantiated in vivo. The current protocol is an evaluation of platelet function in patients undergoing short surgical procedures requiring simple anesthetic agents. Blood is drawn pre-operatively, post-anesthesia induction but prior to surgery, during surgery, and one hour post-operatively. Platelet aggregation is then assessed using a standard aggregometer and standard reagents, including ADP, epinephrine and collagen at varying concentrations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06960-02-COB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

NEW DRUG THERAPY OF PREVIOUSLY TREATED ADULT LEUKEMIA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Van Echo, M.D.	Investigator	COB NCI
Others:	Delia Chuiten, M.D.	Special Assistant to Chief Investigational Drug Branch	CTEP NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

None

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INSTITUTE AND LOCATION

NCI, NIH Baltimore, Maryland 21201

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The combination of pyrazofurin plus azacytidine has been employed in 7 adult ANLL patients and 1 patient with CML in blast crises with 1 patient achieving a CR lasting 40 days. The combination has significant toxicity especially myelosuppression and cutaneous. Additional patients are being entered to further define the antileukemia activity of this combination.

To determine the toxicity of the combination of pyrazofurin plus 5-azacytidine in patients with previously treated malignant disease and to test the possible therapeutic effectiveness of this combination in adult patients with refractory acute leukemia.

The combination was chosen because pyrazofurin has been shown to increase the uptake of 5-azacytidine into human leukemic cells. Initially, the combination was to be given with starting doses of 5-azacytidine 100 mg/m^2 x 5 in three divided doses and pyrazofurin 15 mg/m^2 as a single dose daily x 5. However, the initial two patients who had metastatic colon carcinoma developed severe skin toxicity characteristic of pyrazofurin by the second day of treatment. One of these patients was again re-treated with the combination keeping the azacytidine dosage unchanged and the pyrazofurin reduced 50%. However, he again had significant skin toxicity before the therapy was stopped on the fourth day. Because of this experience it was felt that the dose of pyrazofurin was too high and that the toxicity seen was not secondary to azacytidine. Subsequently, seven patients with acute non-lymphocytic leukemia refractory to conventional agents and one patient with chronic myelogenous leukemia in blast crisis were treated with the combination at a dose of azacytidine 200 mg/m^2 daily x 5 in three divided doses and pyrazofurin 7.5 mg/m^2 as a single dose on the first day of treatment. So far these seven leukemic patients have received twelve courses. The first patient died of overwhelming fungal infection on day 3 and is unevaluable for toxicity or response. The second patient achieved a complete bone marrow and pheripheral blood remission after one course and remained in remission for 40 days. This patient has been given the combination again as a consolidation course but has since relapsed and is currently receiving Phase I agents. The next four patients while having significant bone morrow aplasia induced by the chemotherapy nevertheless had return of their leukemia after two courses. The last patient has developed a significant bone morrow aplasia and has not returned to normal bone morrow cellularity for an evaluation to be made. Myelosuppression is significant with a mean WBC nadir in the range of 500 to 1000, cumm^3. The marrow aplasia may last 6 to 7 weeks. Platelet toxicity is difficult to evaluate because of the heavily treated nature of these patients and the fact that the bone marrows are packed with leukemia. All patients are receiving frequent platelet donor transfusions. Non-hematologic toxicity has included a severe skin rash in all patients. The rash appears on the second day of treatment, it may start anywhere on the body but typically seems to start as a ring area on the posterior aspect of the elbows. Intially it is maculopapular and then becomes confluent encompassing areas several centimeters in expanse. It is non-prutitic but may become painful as it evolves over several days to a week. The rash reaches a maximum on day 3 or 4 therapy and persists for up to 10 days. It may resolve and leave hyperpigmented areas. Other non-hematologic toxicities have included significant nausea and vomiting which is to be expected with any combination with azacytidine; myalgias, weakness, loss of appetite, all of which occurred during the time the drugs were being given and resolved subsequently. One patient has developed lower extremity parathesias idential to that of vincristine neurotoxicity. This patient has received two or three doses of conventional vincristine therapy several months before receiving this combination and had no toxicity at that time.

The study is still open to patient entry and it is anticipated that six to twelve further patients with acute leukemia should be entered to determine the therapeutic efficacy of this combination. Attempts to increase the dose of pyrazofurin to 15 mg/m^2 on subsequent courses have resulted in increased skin toxicity and mucocitis and because of this experience we are unable to increase the dosage further.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06970-01 COB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cis-Dichlorodiammine Platinum II Neurotoxocity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Stanley Ostrow, M.D.	Clinical Associate	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	Carol Koski, M.D.	Assistant Professor Neurology	
	Merrill Egorin, M.D.	Clinical Associate	LCB NCI
	Andre LeRoy, Ph.D.	Biomedical Engineer	

COOPERATING UNITS (if any)

University of Maryland Hospital, Department of Neurology, Biomedical Engineering and Instrumentation, Division of Research Services Branch, NIH

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NCI, NIH Baltimore, MD 21201

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cis-dichlorodiammine platinum II (DDP) an investigational antitumor agent in clinical trials since 1972 has antitumor activity in genitourinary neoplasms, sarcomas, lymphomas and breast cancer. Renal, hematologic, gastrointestinal and audiologic toxicities have been seen.

Recently there have been reports of neurologic toxicity, primarily peripheral neuropathy. In patients treated with cis-platinum at doses of 50 to 100 mg/m^2 every three weeks alone or with other chemotherapeutic agents we have observed sensorimotor polyneuropathics of the hands and feet and cases of papillidema, retrobulbar neuritis and normal pressure hydrocephalus.

PHS-6040
(Rev. 10-76)

With the cooperation of Dr. Koski, Assistant Professor of Neurology at the University of Maryland School of Medicine and Dr. Andre Leroy of the Biomedical Engineering Branch, NIH we have undertaken neuropathological, biochemical and electrophysiologic studies on patients receiving cis-platinum.

In some cases, neurologic toxicity has necessitated discontinuance of platinum therapy. Cessation of therapy has resulted in improvement in neurologic symptoms in most cases. Selected patients who have developed neuropathy on platinum will undergo sural nerve biopsy (small sensory nerve in the leg). Electronmicroscopy and atomic absorption spectrometry will be performed on the biopsied nerve.

Sural nerve biopsy has been performed on one patient to date. Preliminary E-M studies have shown that platinum has appeared to be localized to the endoplasmic reticulum of the nerve.

Prospective studies utilizing serial nerve condition are being performed during the time that patients are receiving platinum therapy in an effort to detect subclinical neuropathy.

Postmortem analysis of nerves in patients who have expired after platinum therapy will also be undertaken.

Publications:

Rhinestein Leon, Ostrow, Stanley and Wiernik, Peter H: Serial nerve conduction studies in Cis-platinum (II) DDP Peripheral neuropathy - Amer. Assoc. of Physical and Rehabilitation Medicine, abstract (submitted).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06971-01 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Phase II Study of Dianhydrogalactitol and Piperazinedione in malignant brain tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Pilar Espana, M.D.	Visiting Scientist	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)
None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Phase II studies of dianhydrogalactitol (14 patients) and piperazinedione (22 patients) have been undertaken in patients with malignant gliomas. All patients were subjected to maximal surgical resection and post operative cranial irradiation prior to drug therapy. Both drugs have myelosuppression as their major side effect, but neither appears to represent a significant improvement of the treatment of these tumors.

PHS-6040
(Rev. 10-76)

Malignant gliomas have a rather uniform and rapidly fatal course. Chemotherapy with various agents has failed to yield evidence of marked palliation.

A. Dianhydrogalactitol (DAG) is the most active of 177 agents tested a mouse ependymoblastoma tumor system. Approximately 30% of administered DAG enters the brain tissue compartment, making this drug promising for therapeutic evaluations in human brain tumors.

Fourteen patients with biopsy proven malignant astrocytoma were evaluated for response and toxicity following maximal surgical resection, post operative radiation therapy (6000 rads to the entire cranium) and Dianhydrogalactitol. Dianhydrogalactitol was given at a dose of 150 mg/m^2 over 1 hour, every 21 days. Dosage was reduced to 130 mg/m^2 for significant myelosuppression and the dose was escalated to 170 mg/m^2 if little or no toxicity was seen on the first treatment. Nine of 14 patients had improvement in neurologic symptoms following this combination treatment while five had definite progression of disease after institution of Dianhydrogalactitol. Correlation with cranial CAT scans was poor, as only 1 patient with clinical improvement had significant mass reduction on CAT scan. The major toxicity of DAG was moderate myelosuppression. The median survival of 28.5 weeks from the start of DAG treatment (range 15-79 weeks) and 72 weeks from diagnosis (range set 19-208 weeks) are not significantly different from other therapeutic reports with this disease.

B. Piperazinedione:

Piperazinedione was found to be active in the intracranially implanted ependymoblastoma tumor system which makes this drug of potential value in the treatment of malignancies in the human central nervous system. Twenty-two patients were with malignant gliomas were treated with maximal surgical tumor resection, followed by post operative radiation therapy (6000 rads to the cranial vault) followed by Piperazinedione, 12 mg/m^2 IV every 21 days in patients with good bone marrow reserve. Patients with inadequate bone marrow reserve were treated at a starting dose of 9 mg/m^2 every three to four weeks. Only 5 of the 22 patients had neurologic improvement lasting 17-73 weeks. The median survival of the overall group from the start of Piperazinedione was 24.5 weeks (range 2-78 weeks). The major toxicity was moderate to severe myelosuppression. These results do not differ remarkably from those of other therapy for this disease.

References:

1. Espana, P, Wiernik PH, and Walker MD: A Phase II study of 1,2:5,6-dianhydrogalactitol (DAG) in malignant glioma. Cancer Treat Rep in press, 1978.

2. Espana P, Wiernik PH, and Walker MD: Phase II study of piperazinedione in malignant brain tumors. Cancer Treat Rep (submitted for publication)

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PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
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PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06973-01 COB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

PHASE I STUDIES OF NEW DRUGS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David Van Echo, M.D.	Investigator	COB NCI
Others:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	Linda Edwards, R.N.	Chemotherapy Nurse	COB NCI
	Mary Scoltock, R.N.	Chemotherapy Nurse	COB NCI

COOPERATING UNITS (if any)

Lab. of Chem. Pharm., NCI; Medicine Branch & Pediatric Oncology Branch, NCI; Medical Oncology Branch of Washington V.A. Hospital

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The result of this project indicates that:

1) the MTD of AMSA when given as a single dose every two weeks will be 70-90 mg/m^2 and that

2) the MTD for PALA when given daily for 5 consecutive days has not been reached.

PHS-6040
(Rev. 10-76)

PROJECT A

To determine the toxicities of m-AMSA (NSC-249992) utilizing an intermittent dosage schedule every two weeks.

AMSA has been given to 21 patients with refractory solid tumors to access its toxicities in man. The drug was given as a 30 minute infusion diluted in 150 cc's of D_5W every 2 weeks for 3 doses. The initial starting dose was 1/3 of the T.D.L. in dogs (the most sensitive animal species) or 10 mg/m^2. Patients were entered in cohorts of 3 or more to each of the following dosage levels; 10, 20, 30, 50, 70, 90, 110 mg/m^2. Except for the patients at the initial level who received one dose at 10 mg/m^2 and all subsequent treatments at 20 mg/m^2, no patient was escalated. Significant toxicities have included: N & V (15%), parethesias (15%) and myelosuppression (only at or above 70 mg/m^2). It is felt that the MTD has been reached on this schedule (single dose every 2 weeks x 3) and will probably be 70 to 90 mg/m^2. Dose limiting toxicity is myelosuppression. No evidence of hepatic, renal, mucosal, or CNS toxicity was observed. Confirmation will be made when the patients at the higher two dose levels complete their courses of therapy (4 more weeks). No meaningful tumor responses were seen.

In cooperation with the Laboratory of Chemical Pharmacology, NCI, determinations were made of serum levels of free AMSA utilizing a fluorescence technique.

Study closed to patient entry and Phase II studies with AMSA in specific tumor types are about to begin.

PROJECT B

To determine the maximal-tolerated dose of PALA (NSC-2224131) in man when given daily for 5 days.

PALA has been given to 11 patients with refractory solid tumors. The initial starting dose was 1/3 of the TDL in dogs (the most sensitive animal species) or 100 mg/m^2/dx5 with courses repeated every 21 days. Patients have been entered in cohorts of 3 or more to each of the following dosage levels; 100, 200, 350 mg/m^2dx5. No patient will be escalated. To date there has been no significant toxicities observed nor any tumor responses.

The study is still in its early stages and it is expected that at least 12-16 further patients will be entered before the MTD is defined for the daily x 5 schedule.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06974 01 COB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

WHOLE BODY HYPERTHERMIA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Van Echo, M.D.	Investigator	COB NCI
Others:	Paul Chang, M.D.	Senior Investigator	COB NCI
	Stanley Ostrow, M.D.	Clinical Associate	COB NCI
	Margaret Whitacre, R.N.	Hyperthermia Nurse	COB NCI
	Nicholas Bachur, M.D.,Ph.D.	Chief, Laboratory of Clinical Biochemistry	LCB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	Renee Gelber, M.D.	Assistant Professor of Surgery, University of Maryland Hospital	
	James W. Ryan, M.D.	Clinical Director, Nuclear Medicine University of Maryland Hospital	

COOPERATING UNITS (if any)

Laboratory of Clinical Biochemistry, BCRC, NCI
Departments of Surgery and Nuclear Medicine,
University of Maryland School of Medicine

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

A method has been developed utilizing equipment provided by the NASA to induce whole body hyperthermia to 41.8°C with minor morbidity to patients. Studies are currently in progress to assess the toxicities of adriamycin plus hyperthermia in the treatment of soft tissue sarcomas and it is planned that a randomized clinical trial will be undertaken assessing the combination of hyperthermia plus adriamcin versus adriamycin alone in the treatment of soft tissue sarcomas.

PROJECT DESCRIPTION: Z01 CM 06974 01 COB

To develop a method to safely induce whole body hyperthermia to 41.8°C and to employ this modality combined with adriamycin for the treatment of soft tissue sarcomas.

A method has been developed utilizing equipment provided by the National Air and Space Administration to safely raise the core temperature of patients to 41.8°. The method employs a water profused nylon mesh and vinyl suit, which encloses the patient from head to ankle. The water temperature is regulated by a control unit utilizing a three liter water bath which is able to control the water temperature from 10° to 110°F. To date two patients with end stage sarcoma have been entered on study and together have completed a total of 14 treatments, 8 of which were at the level of 41.8° for 2 to 4 hours. During this initial development stage, toxicities observed in the patients have been miminal and have consisted of profound weakness which persists for 2 to 4 days following a treatment, circumoral herpes simplex which appeared on both patients at their third treatment and disappeared after five days, transcient rises in liver function tests in one patient, and hypocalcemia for 6 to 12 hours following two treatments in one patient. Both patients are now off study, one because of progressive disease and the other patient because of gram negative sepsis. Before being taken off study, one of the patients had baseline cardiac studies including peripheral vascular resistance, arterial to venous shunting, cardiac output and continuous measurements of mean arterial pressure, right ventricular pressure and pulmonary arterial pressure and pulmonary wedge pressure. Following this one week later he was then monitored in the same fashion and measurements were made of the pharmakinetics of a 10 mg/m^2 dose of IV bolus adriamycin given at the point when the patient first achieved the maximal temperature.

The prototype equipment furnished by NASA has proven to be quite efficient and extremely versatile. However, there is a tendency for the vinyl tubing in the suits to develop leaks which has necessitated the return of some of the equipment to the manufacturer.

When all equipment is fully operational it is our intention to begin a study assessing the toxicities of the combination of adraimycin plus whole body hyperthermia in patients with soft tissue sarcoma and following this, a randomized clinical trial to determine the clinical efficiency of hyperthermia plus adriamycin versus adriamycin alone in the treatment of these malignancies. It is also the intention of this study to measure the clinical pharmakinetics of adriamycin during hyperthermia and immunologic effects of this mode of treatment.

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PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Treatment of Non-Oat Cell Carcinoma of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner, M.D.	Senior Investigator	COB NCI
Others:	Clarence L. Fortner, M.S.	Head, Clinical Research Pharmacy Section	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	Frank Calia, M.D.	Head, Department of Medicine Loch Raven Veterans Administration Hospital	

COOPERATING UNITS (if any)

Department of Medicine, Loch Raven Veterans Administration Hospital

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A large proportion of patients with non-oat cell lung cancer present with surgically unresectable disease and disease outside radiotherapy ports. Although a large number of single drugs have been evaluated in the treatment of non-small cell lung cancer, in general most single agents have been disappointing. Combination chemotherapy utilizing possibly synergistic drugs may warrant testing in this disease. Cyclophosphamide, adriamycin, and CCNU are all single agents with an established low order of activity in non-small cell carcinoma of the lung, and there are animal data suggesting that these three drugs are synergistic. In addition, vincristine has a low order of activity but has little myelotoxicity. The current pilot study was undertaken in combination with the Baltimore Veterans Hospital to determine if this combination chemotherapy program can augment response, survival, and to develop toxicity data. Four patients have been entered onto the study, and it is much too early to determine response rates or toxicity data.

PHS-6040

Project Description: Z01 CM 06975-01 COB

Advanced non-oat cell carcinoma of the lung with disease outside the realm of surgery and radiotherapy, presents a significant problem, and thus the testing of combination chemotherapy programs in this disease is warranted. Cyclophosphamide, adriamycin, and CCNU are single agents of known low order activity in these tumors, but the three drugs are synergistic with each other. Vincristine in addition has a low order of activity with little myelotoxicity. The current pilot study utilizes a combination of these agents given to patients with advanced disease, and the patients are followed at the Veterans Hospital for response and toxicity.

As of April 1978, four patients have been entered onto the study. It is still too early to determine response rate or survival data.

Proposed Course:

Continue investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06976-01 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Augmentation of Alkylating Agents with DMSO

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner, M.D.	Senior Investigator	COB NCI
Others:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A number of advanced cancers such as renal cell carcinoma, and squamous cell carcinoma of the lung, have proven particularly refractory to chemotherapy. It is therefore warranted to test new agents or new experimental modalities in these tumors. Animal studies suggest that the chemotherapeutic activity of certain alkylating agents can be augmented by the use of oral dimethylsulfoxide (DMSO). Patients with metastatic renal cell carcinoma or squamous cell carcinoma of the lung were therefore treated with DMSO and alkylating agents. Fifteen patients with renal cell carcinoma were treated with DMSO in water and thiotepa. There were no objective responses and DMSO and thiotepa were not useful in this disease. Two patients with squamous cell lung cancer have been started on DMSO in water plus cyclophosphamide. No response or survival data are available in this tumor.

PHS-6040
(Rev. 10-76)

There have been many advances in the drug treatments of a variety of human malignancies. Several tumors such as metastatic renal cell carcinoma and advanced squamous cell carcinoma of the lung have proven relatively refractory to conventional drug therapy. Therefore, it is warranted to test new agents or newer experimental modalities for the treatment of these diseases. It has recently been demonstrated that the anti-tumor activity of several antineoplastic drugs, especially the alkylating agents, can be enhanced by the administration or oral dimethylsulfoxide. Patients with metastatic renal cell carcinoma were therefore given dimethylsulfoxide in water orally, then thiotepa in order to test whether the DMSO might enhance the very low order activity of this drug. In addition, patients with advanced squamous cell carcinoma of the lung were given dimethylsulfoxide in water orally and then treated with intravenous cyclophosphamide to test whether this treatment might increase the permeability of the drug and augment the response that might be seen from cyclophosphamide.

As of April 1978, 15 patients with metastatic renal cell carcinoma, (5 patients with prior chemotherapy, one with prior hormonal therapy and 9 without prior therapy), were given 2% DMSO in water and thiotepa. There were no objective responses, while two patients had stabilizations. DMSO was not felt to have augmented the response rate of thiotepa. However, thiotepa is not felt to be active in this disease. Two patients with advanced squamous cell carcinoma of the lung have been started on 2% dimethylsulfoxide in water followed by intravenous cyclophosphamide to determine if the low order of activity of this drug might be enhanced. It has been too early to determine if there is any augmentation or any effect on survival.

Proposed Course:

Continue investigation.

Publications:

1. Aisner J, Wiernik PH: Thiotepa (NSC-6396) and dimethylsulfoxide (NSC-763) in the treatment of renal cell carcinoma. Cancer Ther Trials (In press) , 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06217-09 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Prevention of Infections in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection Research	COB NCI
Other:	K. Newman	Infection Control Nurse	COB NCI
	L. Love	Clinical Associate	COB NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	COB NCI
	V.M. Young	Head, Research Microbiology Section	COB NCI
	C.L. Fortner	Head, Clinical Research Pharmacy	COB NCI
	J. Bender	Clinical Research Pharmacist	COB NCI

COOPERATING UNITS (if any)
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INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9	6.5	2.5

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Infection remains as a major cause of both morbidity and mortality for patients with various types of cancers. The risk of infection increases as new techniques are introduced into medical practice and as greater numbers of patients are treated with more intensive cytotoxic and immunosuppressive therapy. It is therefore essential in the modern practice of oncology that definitive approaches be designed and evaluated to reduce the opportunities for infection. At the Baltimore Cancer Research Center an intensive infection surveillance program assesses the ongoing infectious disease status of the patients and the Center as a whole. From this data base have grown appropriate basic infection control policies and specific research approaches including evaluation of laminar air flow rooms, simpler means of air filtration and reverse isolation, oral nonabsorbable antibiotics and techniques to prevent axillary and oral infections. These programs are enforced by the infection control nurse. A major program has been the reduction of an environmental source of fungi which had contributed to frequent Aspergillus infections. The net result of these varied approaches has been a very substantial reduction in infections among patients at this institution.

PHS-6040
(Rev. 10-76)

Overall Project Description:

Infection remains as a major cause of both morbidity and mortality for patients with various types of cancers. The risk of infection increases as new techniques are introduced into medical practice and as greater numbers of patients are treated with more intensive cytotoxic and immunosuppressive therapy. It is therefore essential in the modern practice of oncology that definitive approaches be designed and evaluated to reduce the opportunities for infection.

Specific Projects:

(A) Surveillance - The cornerstone of an infection control program is an accurate understanding of the infections presently occurring including the sites and etiologic agents, apparent predisposing factors, routes of acquisition of hospital organisms, and changing patterns of morbidity and mortality. At the Baltimore Cancer Research Center, this is accomplished by the daily rounds of an infection control nurse, twice weekly infectious disease rounds, daily evaluation of all infected or potentially infected patients, and an extensive program of both patient and environmental microbial culturing. The data derived is summarized regularly and utilized to adjust, on a continuing basis, the programs in infection control.

(B) - Basic Infection Control Procedures - The Infection Control Policy Manual has been continually updated with enforcement pursued by the infection control nurse. The following procedures are standard: 1) urinary, intravenous and hyperalimentation catheters are avoided except under extenuating circumstances, 2) all intravenous solutions are prepared in laminar air flow hoods, all bottles and tubing are replaced daily, and butterfly needles are changed everyother day, 3) personal hygiene is emphasized including handwashing and the use of special germicidal hand lotions, 4) all inpatients are seen regularly by the infection control nurse for discussion of preventive techniques, 5) results of patient and environmental surveillance cultures are utilized to direct specific control techniques. These techniques have substantially reduced nosocomial infection.

(C) Oral Nonabsorbable Antibiotics - Controlled trials at the BCRC and elsewhere have indicated the usefulness of oral nonabsorbable antibiotics in reducing the incidence of severe infections arising along the alimentary tract and also in the lungs. Although not due solely to the oral antibiotics, the incidence of bacteremia has fallen from 13.3 per 1000 days to 3.2 per 1000 days over the past 5 years. However, the poor taste and frequent nausea and diarrhea leads to difficulties with compliance and hence efficacy. A series of comparative trials was therefore initiated comparing taste, tolerance, compliance, microbial suppression and infection prevention. A double-blind "tasting" by 31 individuals ranked nystatin (N), gentamicin (G), tobramycin (T), amphotericin B(A) and vancomycin (V) liquid in that order from "good-neutral" to "totally unacceptable".

Sixty-two adult patients with acute leukemia undergoing initial remission induction chemotherapy were randomly allocated to receive, double-blind, either GVN or TVN. Tolerance was essentially equal in the two groups in comparing tobramycin to gentamicin, although the poor taste of vancomycin made tolerance to both regimens minimal. Nevertheless, compliance was good to both regimens with better than 90% of the prescribed doses being taken. When taken regularly, both regimens were excellent at suppressing colonic microorganisms. The two regimens appeared to be similar in their efficacy at microbial suppression. The percent of acquired organisms which were resistant to either tobramycin or gentamicin was equivalent in the two groups but was nearly 30% of all gram-negative bacilli acquired by these patients. Overall, the substitution of tobramycin for gentamicin did not appreciably change efficacy or toxicity of these regimens.

Forty-two consecutive patients with acute leukemia in relapse were randomized to receive, in a single blind evaluation, either GVN or GN. Tolerance was much superior in the GN group because of the absence of the distasteful vancomycin but patients in both groups had approximately equal degrees of compliance (82% of prescribed doses were taken). Total colonic suppression was excellent with GVN when taken regularly and was satisfactory with GN although there was persistent, although minimal, growth of some gram-positive cocci especially Streptococcus faecalis in the GN patient group. Despite the persistance of some organisms in the GN group, the incidence of infection in the two groups was identical when based on risk days at various levels of granulocytopenia. Of particular importance was the observation that the GN patients acquired fewer gram-negative bacilli from the hospital environment than did GVN patients. Although the exact mechanism is not clear, the persistance of "colonization resistance" due to persisting bacteria (perhaps especially anaerobes) is an important concept worth further exploration. Colonization resistance prevents the organisms with which the patient is in transient contact from colonizing the alimentary canal and thus prevents subsequent infection by that organism. Not only were fewer organisms overall acquired by the GN group, but this resulted in a reduction in the acquisition of gentamicin-resistant organisms.

Because of the above results, relapsed acute leukemia patients are now being randomly allocated to receive either GN or trimethoprim/sulfamethoxazole (TMP/SMZ) plus nystatin. The concept in question here is whether or not an oral but systemically absorbed antibiotic (TMP/SMZ) will be as effective as the broad specturm aminoglycoside (gentamicin) in suppressing alimentary tract flora. It is to be hoped in both cases that colonization resistance will persist such that transient organisms acquired from the hospital setting will not be able to colonize the patient and cause subsequent infection.

Patients with oat cell carcinoma of the lung who are to receive intensive myelosuppressive chemotherapy are randomly allocated to receive trimethoprim/sulfamethoxazole or a placebo. This study is a further extension of the studies described above to determine the efficacy (and possible toxicities) of trimethoprim/sulfamethoxazole as a potential prophylactic agent for infection prevention during short-term granulocytopenia.

(D) Evaluation of Povidone-Iodine Swabsticks to Prevent Axillary Lesions - Axillary lesion are common in granulocytopenic patients with acute leukemia. Personal hygiene, avoidance of occlusive antiperspirants and shaving only with an electric razor will partially reduce the frequency of these infections which are most commonly caused by gram-negative bacilli as well as by Staphylococcus aureus. Beginning in November, 1975, all patients with acute leukemia were instructed in the use of povidone-iodine swabsticks to the axillae three times per day. All patients with ANLL during the two years before and the two years after this date have been analyzed as to the incidence of axillary lesions at various levels of granulocytopenia. In similar numbers of patients and similar numbers of days at risk, there were 14 lesions in the patient group prior to the institution of the povidone-iodine axillary swabs and only one lesion since their institution in November, 1975. The latter infection occurred in a patient who had discontinued use of the iodine-containing swab. This simple, inexpensive technique has markedly reduced a previously common cause of infection and morbidity.

Publications:

Schimpff, S.C., Hahn, D.M., Brouillet, D.M., Young, V.M., Fortner, C.L. and Wiernik, P.H.: Infection prevention in acute leukemia: comparison of basic infection prevention techniques, with standard room reverse isolation or with reverse isolation plus added air filtration. Leukemia Res. (in press) 1978.

Hahn,D.M., Schimpff, S.C., Fortner, C.L., Smyth, A.C., Young, V.M. and Wiernik, P.H.: Infection in acute leukemia patients receiving oral nonabsorbable antibiotics. Antimicrob. Ag. Chemother. (in press) 1978.

Murillo, J., Schimpff, S.C., and Brouillet, M.D.: Axillary lesions in patients with acute leukemia: evaluation of a preventive program. Cancer (in press) 1978.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06264-09 COB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Evaluation of the Types and Causes of Infectious Diseases in Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection Research	COB NCI
Other:	J. Aisner	Senior Investigator	COB NCI
	K. Newman	Infection Control Nurse	COB NCI
	L. Love	Clinical Associate	COB NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	COB NCI
	V.M. Young	Head, Research Microbiology Section	COB NCI

COOPERATING UNITS (if any)

J.M. Joseph State of Maryland Department of Health; D. Overholser, D. Peterson, Department of Oral Diagnosis, University of Maryland School of Dentistry; J. Murillo, Division of Infectious Diseases, University of Maryland School of Medicine

LAB/BRANCH

Clinical Oncology Branch

SECTION

Section of Infection Research

INSTITUTE AND LOCATION

NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Infection isthe leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies such as Hodgkin's disease and oat cell carcinoma of the lung. The aims of this project are to catalogue and identify the infections, identify the predisposing factors and evaluate new techniques for more rapid and/or accurate diagnosis of infection. This program has been in progress for over seven years and has allowed meaningful evaluation of the types of infection occurring in different types of tumor patients. Specific problems have been identified such as the nature of hospital acquisition of potential pathogens including bacteria and fungi. This epidemiologic data has allowed appropriate and logical development of infection prevention techniques and application of newer methods of infection diagnosis such that there has been a reduction of infection morbidity and mortality.

PHS-6040
(Rev. 10-76)

Objectives:

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aims of this project are to 1) catalogue and identify the infections manifest both clinically and postmortem, 2) identify the predisposing factors which have contributed to the specific type of infection, 3) evaluate new techniques for more rapid and/or accurate diagnosis of infection.

Beginning July 1, 1970, each patient with suspected infection has been seen by one of the investigators who examines the patient and reviews the chart, both initially and throughout the course of the infection. Detailed records are kept of history, examination, laboratory data, etc. Formal infectious disease rounds are held twice weekly to review each recognized infection, discuss management and evaluate how each infection might have been prevented. New evaluations completed, or in progress, this year are included below.

(A) Hospital acquisition of potential pathogens.

1) Hospital acquisition of bacteria and yeasts - Twice weekly surveillance cultures (nose, gingiva, axilla and perirectum; all morphologically distinct isolates were speciated, Pseudomonas aeruginosa and Staphylococcus aureus were typed) were done on 80 consecutive newly diagnosed patients with acute nonlymphocytic leukemia (ANLL) admitted for initial therapy from 1971-1976. Colonization has been divided into those organisms present on the initial baseline cultures ("endogenous" flora) and those organisms which were not present initially but were latter acquired ("exogenous" flora). The data from this extensive microbiologic evaluation shows that 1) multiple potential pathogens were acquired during hospitalization, 2) bacteremias developed from either the baseline or the acquired colonizing organisms at about the same rate (e.g., 25% of patients colonized with P. aeruginosa at admission subsequently had that serotype in a bacteremia and 16% who acquired P. aeruginosa likewise progressed to a bacteremia), 3) a smaller but substantial portion of bacteremias were caused by organisms not previously found in surveillance cultures, these must also be considered as acquired pathogen bacteremias, and 4) overall, 56% of bacteremias were due to hospital-acquired organisms.

It is concluded that hospital acquisition of organisms plays a major role in determining the types of bacteremia which occur in acute leukemia patients with granulocytopenia. One would predict that interruption of the sequence of acquisition to colonization would reduce the incidence of bacteremia in these patients. That such is the case has been proven at the BCRC by the use of environmental controls and other infection prevention techniques discussed under "Prevention of Infections in Patients with Cancer" (Z01-CM-06717-08 COB).

2) Acquisition of Aspergillus flavus and fumigatus from the Hospital Environment - A sudden increase in the frequency of Aspergillus flavus infections provided a setting to evaluate the epidemiology of this infection.

One hundred twenty-five consecutive patients with ANLL admitted over the previous three years were evaluated with 1) sequential nasal surveillance cultures, 2) clinical and necropsy observation for invasive infection by Aspergillus, and 3) complete chart, microbiology and pharmacy record reviews for periods of granulocytopenia at various count levels and number of days of antibiotic administration throughout induction and maintenance therapy. Results of these evaluations have established the epidemiology of Aspergillus flavus at this institution to follow the following general pattern: The granulocytopenic, febrile patient receives empiric broad spectrum antibiotics which on some occasions will cause the nasal bacteria to be sufficiently suppressed to yield "no growth" nose cultures. At this time, spores of Aspergillus flavus in the environment, should they reach the patient's nose, are able to colonize and probably even infect in the presence of granulocytopenia. In the setting of continued prolonged, profound granulocytopenia, this local colonization (or infection) with Aspergillus can progress to invasive aspergillosis in either the sinuses or pulmonary parenchyma. Three practical observations from this evaluation include: 1) routinely obtained surveillance cultures of the nose can be helpful in detecting the patient with local colonization who is therefore at greater risk of subsequent invasive aspergillosis. 2) Knowledge of the epidemiology suggests methodologies for infection prevention such as reducing the environmental contamination with Aspergillus spores or the use of reverse isolation techniques such as laminar air flow rooms (in which no case of Aspergillus infection has yet been detected). 3) The finding of a nose culture positive for Aspergillus flavus in a clinical setting of new pulmonary infiltrate, granulocytopenia, and fever not responding to antibacterial antibiotics is sufficient presumptive evidence to institute antifungal therapy pending results of aggressive invasive techniques for diagnosis.

(B) Legionnaire's Disease -

With the recent observation that Legionnaire's Disease is more likely to be found in the compromised host who is exposed to the organism, the BCRC reviewed cases of pneumonia of ill-defined etiology in an attempt to determine which, if any, had been caused by Legionnaire's Disease. With the cooperation of the Center for Disease Control, 43 patients with clinically documented pneumonia in whom no etiologic agent could be previously detected were screened by serology for the presence of antibody to the Legionnaire's bacillus. Only one patient had evidence of a titer rise and this patient, on biopsy, had electron microscopic evidence of a unicellular microorganism suggestive of the Legionnaire's disease bacillus. With the recent availability of the immunofluourescent staining technique, tissue sections from many of these pneumonias will be evaluated. Results of the serologic assays, however, suggest that Legionnaire's disease has not been a major cause of undiagnosed pneumonia at this institution in the last few years.

(C) Epidemiology of an Unusual Diphtheroid-Like Organism -

Reports from the NCI in Bethesda, the Johns Hopkins Medical Institutions and the Fred Hutchinson Cancer Center in Seattle have noted the appearance of an invasive diphtheroid-like organism capable of causing severe infections in the compromised host. It has been characterized by resistance to multiple antibiotics. In the fall of 1977, and extending to the current time, 8 patients have been infected with such an unusual diphtheroid and numerous other individuals have been found to be colonized. As discussed elsewhere under the Section of Research Microbiology, techniques were developed to rapidly isolate and identify the organism. Extensive work has been done to characterize this organism as an unusual subtype of Corynebacterium. Epidemiologic studies have indicated that it tends to grow on the skin especially in the groin or other moist areas, that it can colonize the alimentary canal and that in the presence of profound granulocytopenia, this alimentary canal colonization may lead to invasion and sepsis. Only vancomycin has proven to be useful therapeutically. Spread from patient-to-patient has been documented.

(D) Evaluation of Periodontal Hygiene and Subsequent Infection -

Although periodontal and other infections are of considerable concern following cytotoxic therapy or radiation therapy, little is known of the development of such lesions in the cancer patient. In a cooperative study with the Department of Oral Diagnosis at the University of Maryland School of Dentistry, each newly admitted patient with acute nonlymphocytic leukemia, Hodgkin's disease, or oat cell carcinoma of the lung is evaluated by a dentist and a dental hygienist. Following this evaluation and completion of an objective written assessment of oral hygiene, each patient is randomly allocated to routine oral care (brushing and flossing) with or without a complete oral prophylaxis. Appropriate antitumor therapy is then begun and the patient is followed three days per week if an inpatient by the dental hygienist and weekly if an outpatient. Objective assessments are made on a weekly basis and correlated with other clinically derived data including presence or absence of infection. This study is in its early stages and therefore not yet evaluable but should produce useful data with respect to the following questions: What is the status of the periodontum at the time of tumor diagnosis of three different types of tumors (leukemia, lymphoma and solid tumor)? How is the periodontum affected by the disease process, chemotherapy (leukemia and oat cell carcinoma), radiation therapy (Hodgkin's disease) or radiation plus chemotherapy (Hodgkin's disease)? How does the status of the periodontum correlate with subsequent oral infection? Does pre-therapeutic dental prophylaxis affect the natural progression of periodontal disease in association with cytotoxic or radiation therapy?

Publications:

Aisner, J., Schimpff, S.C., Bennett, J.E., Young, V.M., and Wiernik, P.H.: Aspergillus infections in cancer patients. Association with fireproofing materials in a new hospital. JAMA 235:411-412, 1976.

Remington,J.S., Schimpff, S.C., Hughes, W.T., Armstrong, D., Klastersky, J. and Bodey, G.P.: Life-threatening infections in the compromised host. In Proceedings of the 10th International Congress of Chemotherapy, Zurich, 1977, pp. 244-245.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06266-09 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Therapy of Suspected Bacterial Infection in Granulocytopenic Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection Research	COB NCI
Other:	L. Love	Clinical Associate	COB NCI
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	C. L. Fortner	Head, Clinical Research Pharmacy	COB NCI
	J.F. Bender	Research Pharmacist	COB NCI
	J. Aisner	Senior Investigator	COB NCI
	C. A. Schiffer	Senior Investigator	COB NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)

H. Standiford Division of Infectious Diseases, University of Maryland: European Organization for Research on Treatment of Cancer, Antimicrobial Therapy Project Group.

LAB/BRANCH

Clinical Oncology Branch

SECTION

Section of Infection Research

INSTITUTE AND LOCATION

NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.50

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of consecutive antibiotic trials for suspected sepsis in granulocytopenic cancer patients began in 1969, and continues to the present. Initial evaluations proved the usefulness of carbenicillin and gentamicin and later trials, including a large multiinstitutional trial, indicated that carbenicillin (ticarcillin) plus gentamicin had the best efficacy-to-toxicity ratio of the three possible two-drug combinations of carbenicillin (ticarcillin), gentamicin and cephalothin. A high incidence of nephrotoxicity secondary to the combination of cephalothin and gentamicin was noteworthy. In the next trial, cephalothin plus amikacin in 93 consecutive patient-trials was not as nephrotoxic but was more nephrotoxic than any of the following two-drug combinations evaluated in 1976-1978. Ticarcillin plus gentamicin was compared to ticarcillin plus amikacin and ticarcillin plus netilmicin. In 183 consecutive patient-trials, the efficacy was high and the toxicity minimal. With the addition of early granulocyte transfusions for poor risk patients, a response rate of over 90% has been demonstrated in the past one and one-half years.

Project Description:

Despite improvements in preventive techniques, infections remain as one of the most common serious complications of cancer therapy. The BCRC Section of Infection Research has continuing programs designed to improve the therapeutic approach to these infections.

(A) Empiric Antibiotic Therapy for the Febrile Granulocytopenic Patient - The setting of new fever during granulocytopenia represents infection in ∿60% of cases with bacteremia occurring in 20% thus necessitating the prompt empiric use of broad spectrum, bactericidal antibiotics.

1) Ticarcillin, an analog of carbenicillin, has been used in over 300 patient-trials at the BCRC. It has been found to be equally efficacious to carbenicillin but, because it can be utilized at one half the dosage of carbenicillin, has less dose-related side effects. As a result, ticarcillin has supplanted carbenicillin, and ticarcillin in combination with gentamicin has become the standard of therapy to which all other approaches are currently being compared.

2) Ticarcillin plus an aminoglycoside was shown to have the best efficacy-to-toxicity ratio of the various possible two-drug combinations of carbenicillin (or ticarcillin), gentamicin, and cephalothin. The combination, ticarcillin plus gentamicin was equally or more effective than the other possible combinations and the cephalothin plus gentamicin combination had an unacceptably high incidence of nephrotoxicity.

3) Cephalothin plus gentamicin is the most commonly used antimicrobial combination in the United States today but results of the BCRC prospective evaluation and two concurring randomized trials have indicated that this combination has a high degree of nephrotoxic potential particularly in the older patient with reduced renal function. Cephalothin plus tobramycin has also been shown in a randomized trial to have a high incidence of nephrotoxicity but our own evaluation of cephalothin plus amikacin would suggest a relatively low order of serious nephrotoxic potential for this combination.

4) Utilizing ticarcillin plus gentamicin as the basis for comparison, ticarcillin plus amikacin and ticarcillin plus netilmicin were evaluated in a three-way prospective randomized trial of granulocytopenic cancer patients with fever. Amikacin was chosen because multiple reports indicate that fewer gram-negative bacilli are resistant to amikacin than to gentamicin. Netilmicin was chosen because early animal studies indicated a reduced level of nephrotoxicity. Following randomization of 183 consecutive febrile episodes, it was found that the three regimens are equally efficacious and equally nontoxic. The response rate for bacteremias was approximately 80% and was essentially 100% for all other microbiologically or clinically documented infections. There were approximately equal numbers of aminoglycoside-resistant organisms, although there were fewer amikacin-resistant *Pseudomonas aeruginosa*. For gram-negative bacteremias, antibiotic susceptibility to both assigned antibiotics was the key to successful outcome: Twelve of 12 bacteremias in which the organism was susceptible

to both antibiotics improved, whereas only 9 of 13 in which the organism was susceptible to only one of the two antibiotics improved. Nephrotoxicity was minimal with only four patients having a minimal and nonclinically important rise in creatinine during or immediately following therapy attributed to the protocol antibiotics. This study has further augmented the observation that early empiric therapy with ticarcillin plus an aminoglycoside is effective and has shown that each combination is relatively nontoxic but that the choice of aminoglycoside depends upon known patterns of microbial resistance in any given institution.

5) In order to further evaluate the potential for cephalosporin-aminoglycoside nephrotoxicity and to determine whether or not the addition of a cephalosporin to the ticarcillin-aminoglycoside regimen would improve efficacy, a multiinstitutional trial was begun comparing ticarcillin plus amikacin to ticarcillin plus amikacin plus cefazolin. As of this time, 80 BCRC patient-trials have been entered to the group study. Nephrotoxicity has been minimal in both groups suggesting that the ticarcillin in the three-drug combination may act as an osmotic diuretic to help prevent aminoglycoside-cephalosporin associated nephrotoxicity.

6) Sera from 32 patients receiving either ticarcillin plus cephalothin (T+C), ticarcillin plus gentamicin (T+G), or cephalothin plus gentamicin (C+G) were obtained one hour post antibiotic administration and examined for bactericidal activity against 11 strains each of the most common pathogens infecting the granulocytopenic host: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylcoccus aureus*. Each of the three antibiotic regimens produced a high degree of bactericidal activity in these sera against *S. aureus* and *E. coli*. *P. aeruginosa* was equally, although poorly, killed by sera containing ticarcillin (T+G=T+C), whereas cephalothin plus gentamicin produced no measurable serocidal activity. Sera with cephalothin plus gentamicin killed *K. pneumoniae* more effectively than ticarcillin plus gentamicin; ticarcillin plus cephalothin produced the least cidal effect of the three regimens against this organism. The bactericidal activity of the serum of these 32 patients supplemented the overall clinical observations suggesting that ticarcillin plus gentamicin is a useful initial regimen for empiric therapy of febrile episodes in granulocytopenic cancer patients.

7) Ticarcillin and gentamicin sera levels were measured one and five hours after intravenous administration of antibiotics to 64 and 55 patients respectively. Patients receiving ticarcillin plus gentamicin had a significantly lower mean gentamicin level one and five hours after antibiotic administration than patients receiving cephalothin plus gentamicin. The serum ticarcillin levels were significantly lower five hours after administration in patients receiving ticarcillin plus gentamicin than those receiving ticarcillin plus cephalothin. These unexpectedly low antibiotic serum levels in patients with serious infections treated with these antibiotic combinations are of potential clinical significance and indicate the need to determine serum antibiotic concentrations (especially of the aminoglycoside) in patients with normal creatinines while being treated with combinations such as ticarcillin plus gentamicin.

(B) Evaluation of New Antibacterial Antibiotics

1) In a series of investigations outlined elsewhere under the Section of Research Microbiology, newly developed antibiotics are being evaluated in vitro. The aminoglycosides, gentamicin, tobramycin, amikacin and netilmicin have been found to be approximately equivalent in their activity with the exception that fewer strains of Pseudomonas aeruginosa are resistant to amikacin than to the other three aminoglycosides.

2) In synergy studies with Dr. Harold Standiford at the University of Maryland Hospital, Division of Infectious Diseases, a series of laboratory evaluations have proven the reliability of a simple technique to determine clinically useful synergy between semisynthetic penicillins such as carbenicillin or ticarcillin and aminoglycosides such as gentamicin, tobramycin, and amikacin. For Pseudomonas aeruginosa, synergism will be present between the semisynthetic penicillin and the aminoglycoside if the minimal inhibitory concentration (MIC) is less than 64 µg/ml and provided the MIC to carbenicillin or ticarcillin is less than 1600 µg/ml. Both of these levels can be easily detected by observing the growth pattern around the Kirby-Bauer disc; if growth comes up to the disc of either antibiotic then synergy will not occur. However, if there is any rim around both discs then clinically useful synergy will occur even if the amount of growth around the disc would normally indicate "resistance" to the microorganism in question. This simple laboratory test will indicate whether or not the carbenicillin-aminoglycoside synergistic potential exists even when the Kirby-Bauer disc method would suggest aminoglycoside resistance.

3) A similar evaluation utilizing cephalothin plus aminoglycosides against Klebsiella pneumoniae indicates that approximately two-thirds of randomly selected strains from clinical specimens will show synergy between cephalothin plus any of the commonly used aminoglycosides. However, the simple laboratory test indicated above as useful for Pseudomonas aeruginosa does not predict for Klebsiella pneumoniae. Synergy to this organism can only be detected through the use of the standard, and complex, checkerboard technique.

4) Three new semisynthetic aminoglycosides have become available for investigational use. Piperacillin, mezlocillin and azlocillin are substantially more effective in vitro than ticarcillin or carbenicillin against clinical isolates of Klebsiella pneumoniae, Streptococcus faecalis, Staphylococcus aureus, Serratia marcescens and Escherichia coli. In each of these instances, piperacillin was most active followed by mezlocillin followed by azlocillin. For Pseudomonas aeruginosa, piperacillin is most active followed by azlocillin followed by mezlocillin which is equivalent to ticarcillin, followed by carbenicillin.

Results of the above laboratory evaluations led to our interaction with the multiinstitutional group to determine the value of the ticarcillin plus amikacin plus cefazolin combination to ticarcillin plus amikacin to determine whether the addition of cefazolin would be more useful for Klebsiella pneumoniae. Clinical studies to begin shortly include evaluations of mezlocillin and piperacillin for febrile episodes in granulocytopenic cancer patients. The broad spectrum of activity of these latter two antibiotics suggests the potential for their use as single agents without the need for the accompanying aminoglycoside. If such an approach is proven to be effective, not only the cost, but the potential of aminoglycoside nephrotoxicity would be reduced substantially.

Publications:

EORTC International Antimicrobial Therapy Project Group: Three Antibiotic Regimens in the Treatment of Infection in Febrile Granulocytopenic Patients with Cancer. J. Inf. Dis. 137:#1 14-29, 1978.

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Hahn, D.M., Schimpff, S.C., Young, V.M., Fortner, C.L., Standiford, H.C. and Wiernik, P.H.: Amikacin and cephalothin: Empiric regimen for granulocytopenic cancer patients. Antimicrob. Ag. Chemother. 12: 618-623, 1977.

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Levi, J.A., Schimpff, S.C., Slawson, R.G., and Wiernik, P.H.: Evaluation of radiotherapy for localized inflammtory skin and perianal lesions in adult leukemia: A prospectively randomized double blind study. Cancer Treat. Reports 61: 1301-1305, 1977.

Murillo, J., Standiford, H.C., Schimpff, S.C., Tatem, B.A.: Comparison of serum bactericidal activity among three antimicrobial combinations. Antimicrob. Ag. Chemother. (in press), 1978.

Schimpff, S.C. and Aisner, J.: Empiric antibiotic therapy. Cancer Treat. Rep (in press) 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06950-02 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Therapy of Fungal Infections in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner	Senior Investigator	COB NCI
Others:	Stephen C. Schimpff, M.D.	Head, Section of Infection Research	COB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Section of Infection Research

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fungal infections in cancer patients are difficult to diagnose and such diagnoses are frequently only made at necropsy. Aspergillus infections were common at the BCRC (see #6264 regarding prevention) and were usually fatal, but as diagnostic procedures improved, earlier therapy became possible. Therefore, 17 consecutive patients with documented aspergillosis were reviewed to determine the impact of earlier diagnosis and prompt treatment with amphotericin B. Sixteen of the 17 had hematologic malignancies, and all had marked granulocytopenia. Six patients were diagnosed and treated within 96 hours of the appearance of infiltrates; three had complete resolution and three had a partial response to therapy despite continued granulocytopenia. Thus, aggressive diagnostic techniques are warranted. We are now evaluating the addition of rifampin to amphotericin B, known to be synergistic against some fungi in animal models, for early therapy. Thirty-five patients have been randomly allocated to date to amphotericin B rifampin, but after stratification for the various fungal infections, there are too few patients from which to draw meaningful conclusions other than that early application of therapy continues to be the best approach to infection resolution.

Project Description Z01 CM 06950-02 COB

Infections with *Candida* sp, *Torulopsis glabrata*, *Aspergillus* sp and *Phycomycete (Mucor)* sp are increasing as more patients survive initial bacterial infections. Unfortunately, amphotericin B is the only antibiotic of defined efficacy yet the present response rate is low. Recently rifampin has been shown in vitro to be synergistic with amphotericin B against these yeast and fungal organisms. The BCRC has therefore begun a prospective randomized trial of amphotericin B versus amphotericin B plus rifampin as initial therapy for proven or strongly suspected fungal infection. The study will continue.

A) Early Therapy of Invasive *Aspergillus* Infection - *Aspergillus* infections in cancer patients are difficult to diagnose and such diagnoses are frequently made at necropsy. Earlier therapy has been proposed to provide better response. Therefore, 17 consecutive patients with documented aspergillosis were reviewed to determine the impact of earlier dianosis and prompt treatment with amphotericin B. Sixteen of the 27 had hematologic malignancies, and all had marked granulocytopenia. Six patients were diagnosed and treated and all had marked granulocytopenia. Six patients were diagnosed and treated within 96 hours of the appearance of infiltrates. Three of these 6 had complete resolution of all signs and symptoms of *Aspergillus* infection. The other three had a partial response to therapy despite continued granulocytopenia. All 11 patients in whom antifungal therapy was either delayed (6) or not given (5) for at least 2 weeks after the infiltrate was present, died with progressive aspergillosis. Aggressive diagnostic techniques to establish the diagnosis of aspergillosis are warranted so that early institution of antifungal therapy can be instituted, which may then be successful in resolving these potentially fatal infrections. This study has been completed and has lead to the therapeutic evaluation described below.

B) Evaluation of Rifampin in Combination with Amphotericin B - Infections with *Candida* sp, *Torulopsis glabrata*, *Aspergillus* sp and *Phycomycete (Mucor)* sp are increasing as more patients survive initial bacterial infections. Unfortunately, amphotericin B is the only antibiotic of defined efficacy yet the present response rate is low. Recently rifampin has been shown in vitro to be synergistic with amphotericin B against these yeast and fungal organisms. The BCRC has therefore begun a prospective randomized trial of amphotericin B versus amphotericin B plus rifampin as initial therapy for proven or strongly suspected fungal infection. Thirty-five patients have been randomized to date (17 to amphotericin B alone and 18 to amphotericin B plus rifampin); after separation into various types of fungal infections, this represents too few patients to show any difference, should a difference exist, between the two regimens. The study will continue.

Publications:

Aisner, J., Schimpff, S.C., and Wiernik, P.H.: Treatment of invasive aspergillosis: relation of early diagnosis and treatment to response. *Ann. Intern. Med.* 86:539-543, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06951-02 COB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Infection Research in Oncology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection Research	COB NCI
OTHER:	L.J. Love	Clinical Associate	COB NCI
	J. Aisner	Senior Investigator	COB NCI
	K. Newman	Infection Control Nurse	COB NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	COB NCI
	V.M. Young	Head, Research Microbiology Section	COB NCI
	C.A. Schiffer	Senior Investigator	COB NCI

COOPERATING UNITS (if any)

J. Murillo; H. Standiford; R. Hornick Division of Infectious Diseases, University of Maryland Hospital

LAB/BRANCH

Clinical Oncology Branch

SECTION

Section of Infection Research

INSTITUTE AND LOCATION

NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aim of this program is an integration of research, education, and patient care with regard to the infections arising in patients with cancer. The research areas include infection epidemiology, diagnosis, prevention and treatment. Education includes the teaching of BCRC clinical associates the fundamental problems and techniques for infection management, teaching the nursing staff basic prevention and treatment modalities, and teaching the University of Maryland Infectious Disease fellows and students concepts of infection control in the tumor patient. Patient care includes patient education in infection prevention in addition to infection diagnosis, prevention and management.

PHS-6040
(Rev. 10-76)

The frequency and severity of infection in patients with advanced cancer necessitates an ongoing program of 1) infection research, 2) education and 3) patient care. The aims of this program are to integrate these three areas into the ongoing clinical and laboratory programs at the BCRC.

Research areas include infection epidemiology, diagnosis, prevention and therapy (see #6264, #6266, #6950, and #6217).

Education includes the training of BCRC clinical associates, University of Maryland Infectious Disease fellows, and Department of Medicine rotating residents in the management approaches necessary for optimum care of the infected or potentially infected cancer patient. This is done through the daily work rounds plus informal discussions. Nursing education, done largely by the Infection Control Nurse, is designed around twice monthly teaching conferences plus informal patient management discussions.

An integrated team approach has been developed, based around twice weekly formal infection rounds, and includes active participation by physicians from the Infection Research Section and the Cell Support Program, the infection control nurse, microbiologist, pharmacist, and attending physicians. This approach ensures thorough discussion of the various management approaches for each patient while reviewing the requirements of protocol studies.

Publications:

Schimpff, S.C.: Infection in the compromised host. In Principles and Practices of Infectious Diseases, (Ed.) J.E. Bennett, John Wiley and Sons, Inc., Publ. New York, New York, (in press), 1978.

Schimpff, S.C.: Infections in patients with acute leukemia with emphasis on infections associated with granulocytopenia. In Principles and Practices of Infectious Diseases, (Ed.) J.E. Bennett, John Wiley and Sons, Inc., Publ. New York, New York (in press), 1978.

Schimpff, S.C.: Hodgkin's disease. In Principles and Practices of Infectious Diseases, (Ed.) J.E. Bennett, John Wiley and Sons, Inc., Publ. New York, New York, (in press), 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06988-01 COB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Radiation-Related Thyroid Dysfunction in Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection Research	COB NCI
Other:	C.H. Diggs	Senior Investigator	COB NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	COB NCI
	P. Salvatore	Secretary, Section of Infection Research	COB NCI

COOPERATING UNITS (if any)
J.G. Wiswell Section of Endocrinology, Department of Internal Medicine, University of Maryland Hospital and School of Medicine

LAB/BRANCH
Clinical Oncology Branch

SECTION
Section of Infection Research

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS: 0.25	PROFESSIONAL: 0.125	OTHER: 0.125

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Late complicationsin the treatment of Hodgkin's disease with radiation therapy are increasingly being recognized. Thyroid dysfunction following irradiation is one such complication which has not been widely recognized, probably because of the subtle clinical nature of minor thyroid hormone imbalance. In addition, it has long been thought that the thyroid was relatively resistant to radiation therapy. Two hundred thirty-two patients with Hodgkin's disease followed at the BCRC have been evaluated for post-therapy thyroid dysfunction. Thirty-nine percent of those with mantle radiation therapy had an elevated TSH, but normal T_4, but an additional 26% had an elevated TSH plus a depressed T_4. Thus, 65% of 184 patients had evidence of thyroid dysfunction following mantle radiation therapy. Since thyroid hormone replacement is simple, inexpensive and usually without major side effects if properly monitored, it is reasonable in light of the foregoing to place all patients with an elevated TSH on thyroid replacement therapy whether or not they are chemically hypothyroid.

Project Description:

Two hundred thirty-two patients with Hodgkin's disease returning to the Baltimore Cancer Research Center for routine clinic visits had a serum sample obtained for thyroid function tests. Some patients had multiple serial serum samples taken over many months. Serum thyroxine (T_4), T_3 uptake by thyroxine binding globulin, the effective thyroxine ratio and the serum thyroid stimulating hormone (TSH) were measured. Patients with stages I-IIIA received radiation therapy or radiation plus adjuvant chemotherapy and patients with stages IIIB-IV received chemotherapy alone. Radiation therapy was delivered from a cobalt 60 source with midplane dose of approximately 4000 rads given as 200 rads per day, five days per week for four weeks weighted to give two-thirds of the dose from the anterior port and one-third through the posterior port.

Major Findings:

Of 184 patients who received mantle radiation therapy, 72 (or 39%) were found to have an elevated TSH but normal thyroid hormone level. An additional 48 patients (26%) had an elevated TSH plus an abnormally low T_4. Combined, 120 of 184 patients (65%) had evidence of thyroid dysfunction following mantle radiation therapy. Among the 48 patients who had not received mantle radiation therapy, 8 (17%) had an elevated TSH but none had depressed thyroid hormone levels.

With an incidence of 26% of patients developing chemical hypothyroidism within a few years after radiation therapy, it is clear that this is a major complication of irradiation for Hodgkin's disease. All such patients should therefore have TSH determinations done every six months for the first five years following irradiation with determination of thyroxine levels should the TSH be elevated. Replacement therapy with sodium levothyroxine will usually be sufficient. For those patients with elevations of TSH alone, it is appropriate to begin replacement therapy for this state of compensated hypothyroidism. Patients may benefit symptomatically, the lessened production of TSH will prevent the rare occurence of sella turcica damage, and, since elevated TSH in the presence of radiation damaged thyroid tissue is known to be carcinogenic, TSH suppression may lessen the potential for later development of thyroid carcinoma.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06027-09 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Microbiological Services for Clinical and Research Studies of Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology Section	CL NCI
Others:	Marcia R. Moody, Ph.D.	Research Microbiologist	CL NCI
	Arthur S. Levine, M.D.	Chief, Ped. Oncology	COP NCI
	Phillip A. Pizzo, M.D.	Senior Investigator	COP NCI
	Norman R. Friedman	Microbiologist	CL NCI
	Michael T. Hargadon	Microbiologist	CL NCI
	Maureen J. Morris	Lab. Scientist	CL NCI
	Stephen C. Schimpff, M.D.	Head, Infections Research	CL NCI

COOPERATING UNITS (if any)
Hazleton Laboratories, Vienna, Va.
Diagnostic Laboratory, Microbiological Associates, Walkersville, Md.

LAB/BRANCH
Clinical Oncology

SECTION
Microbiology

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3 1/2	2 1/2	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study samples from cancer patients to determine the presence of bacteria, mycoplasma, fungi, and viruses which cause infection. Surveillance cultures which are taken upon admission of the patient to the hospital provide a baseline of the patients' flora. Carriers of pathogenic or highly resistant organisms can then be isolated. Repeated surveillance cultures from high risk patients allow determination of hospital acquisition of potential pathogens. Sources of hospital acquired microorganisms can thus be sought and eliminated. Environmental cultures provide further information as to possible sources of hospital-acquired microorganisms through detection of "shedders", efficacy of cleaning procedures, and unexpected contaminated sources. A total of 18,280 patient and environmental samples were cultured during the period of 4/1/77-3/31/78 for the presence of bacteria, mycoplasma, and fungi. In addition, 239 samples were cultured for viruses, 54 electron microscopic studies were performed to detect virus, and 3,624 serum samples were examined to detect rises in antibody titers against viruses. A serum bank which collected 7,872 sera from cancer patients for these and other research purposes were collected.

PHS-6040
(Rev. 10-76)

The purpose of this project is to provide high quality clinical diagnostic microbiologic evaluation of samples collected from cancer patients to determine causes of their infectious processes. This support, together with the determination of susceptibilities of the microorganisms recovered, allows selection of appropriate antibiotic therapy against infection of these patients. Surveillance cultures taken from the patients on admission to the hospital and subsequently serve several purposes: 1) to monitor initial flora so that patients who carry pathogens or resistant microflora can be isolated; 2) to determine acquisition of potential pathogens or resistant organisms so that a) the patient can be isolated, b) the susceptibility of such microorganisms can be determined before the patient becomes infected and c) the source of the organism can be sought and in some instances, eliminated; and 3) to follow in vivo development of resistance to the antibiotics most frequently utilized.

The aerobic, anaerobic bacterial and fungal flora studies of the patient samples and some of the environmental samples were performed by Hazleton Laboratories of America (HLA) under Contract N01-CM-67080. Surveys of patients and personnel for specific microorganisms and many of the environmental samples were performed by personnel of the Microbiology Section. From 4/1/77 to 3/31/78, 18,280 samples were studied by HLA. Viral studies of 239 samples for viral isolation, 54 samples for EM evaluation, and 3,624 serum samples for antibody titer determinations between 2/14/77-2/10/78 were made by Microbiological Associates (MA), Walkersville, Md. under Contract N01-CM-77131. These contracted facilities will no longer be utilized during the next year. The contract with HLA was terminated in June, 1978 and that with MA in August,1978.

This project supplies microbiologic support for a number of protocols; among these are the Pharyngitis protocol, No. 7504; the Herpes zoster protocol, No. 7506; the Empiric Antibiotic Therapy protocol, No. 7712; the Trimethoprim/Sulfamethoxazole protocol, No. 7714, etc. as well as for other investigations and publications. It continues to supply information concerning fungal spores derived from insulating material on the overhead beams (Project No. 06264). All of the data generated by microbiologic examination of patient samples is stored in a computer and frequent retrievals make the data available to physicians for patient care and to investigators for research purposes (See Project No. 06039).

Sera is collected on a regular basis from the cancer patients and stored in a serum bank for later use for diagnostic and research purposes. Pertinent data about the sera, such as the patient's name, hospital number, diagnosis, dates, and amounts, are entered into a computer. Printouts from this program serve to provide not only a rapid, accurate, and easy accessibility, but also informs the physicians as to the availability of sera for diagnostic and research purposes.

These microbiologic studies, essential to patient care, will continue to be provided by the Microbiologic Section without the aid of contracting laboratories.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06039-09 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Computerized Microbiology Reporting and Data Retrieval Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology Section	CL NCI
Others:	John W. Gerster	Computer Specialist	CL NCI
	William L. Robinson	Computer Specialist	CL NCI

COOPERATING UNITS (if any)
Diagnostic Laboratory, Microbiological Associates, Walkersville, Md.
Hazleton Laboratories, Vienna, Va.; Tymshare, Inc., Baltimore, Md.
HSR, Public Health Service Hospital, Baltimore, Md.

LAB/BRANCH
Clinical Oncology

SECTION
Microbiology

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2/3	1/2	1/6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to provide an accurate, rapid, means to report and update preliminary results of clinical microbiologic studies of samples collected from cancer patients and to provide final reports of the data suitable for the patients' charts. The data are generated at two different laboratories located some distance from the hospital and at the laboratories of the Microbiology Section. Preliminary data are sent via telephone lines from the laboratories to a teletype at the BCRC where individual copies are utilized for patient care and a variety of other purposes. Final report data are sent by the laboratories to a computer center from which final reports are generated and which permanently stores the data. Prior to storage, the data is processed through error detection and quality control systems. The data from individual patients are summarized on a regular basis in a form suitable for placement in the patients' charts. Data reviews are available by means of retrieval programs for patient care and research purposes. These retrieval programs regularly provide reviews of antibiotic susceptibilities, changes in prevalence of certain microorganisms, predominance of organisms from a variety of sources, etc.

PHS-6040
(Rev. 10-76)

This project is designed to provide rapid, accurate, preliminary reports of clinical microbiologic studies of samples from cancer patients; this data has been generated principally by two distantly located contracting laboratories. It is sent via telephone lines to a teletype located at the Baltimore Cancer Research Center. Further data are forwarded until the work has been completed. Individual copies of the preliminary reports are: 1) placed temporarily in the patient's chart; 2) posted in a centrally located physicians' area for general review; 3) sent to the Head, Infections Research Section for review; 4) given to the Head, Microbiology Section for review of accuracy and taken to Infections Rounds; and 5) reviewed by various other persons to produce a monthly Infection's Report and to provide immediate protocol information. The completed final reports of the culture data are sent to a computer center where error detection systems are used to assure accuracy before the data are permanently stored. These data are used to generate final reports, to provide summary reports at regular intervals, which are placed in the patients' charts, and to provide a base for retrieval for research purposes.

Retrieval of antibiotic susceptibilities of microorganisms are done regularly to monitor the development of resistance to antibiotics in current use. A recent review to compare disc susceptibilities to minimum inhibitory concentrations revealed that disc susceptibility tests for Klebsiella pneumoniae, when the discs provided by the manufacturer for testing a new semisynthetic penicillin were used (as recommended for other Enterobacteriaceae), were unreliable. Other recent retrievals have reviewed the prevalence of Group D streptococci and Serratia (See project Z01 CM 06219). This data base is also often used by the Infections Research Section for data pertinent to studies on infection and therapy of the cancer patients and by the Microbiology Section to monitor possible transmission of bacteria among patients (See project Z01 CM 06938).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06219-09 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on Cancer Patient Microbial Flora:
Acquisition, Colonization, & Infection

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology	CL NCI
Others:	Marcia R. Moody, Ph.D.	Research Microbiologist	CL NCI
	Michael T. Hargadon	Microbiologist	CL NCI
	Maureen J. Morris	Laboratory Scientist	CL NCI
	Stephen C. Schimpff, M.D.	Head, Infections Research	CL NCI

COOPERATING UNITS (if any)

Hazleton Laboratories, Vienna, Va.
University of Maryland School of Medicine, Baltimore, Md.

LAB/BRANCH
Clinical Oncology

SECTION
Microbiology

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 5/6	1 1/2	1/3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the microbial flora of cancer patients. Surveillance cultures taken from these patients provide a means to monitor the flora and its acquisition. Clinically important strains are subspeciated by means of serotyping, phagetyping, etc. An unusual coryneform bacterium, which was recovered from 20 patients at the BCRC, was studied extensively. This organism was gram positive, non-motile and found to be resistant to all antibiotics tested with the exception of vancomycin, occasionally rifampin, and rarely erythromycin. Group D streptococci were evaluated from surveillance cultures, urine cultures and other sources. Order of prevalence for the various species was established and susceptibilities determined. A study of Serratia marcescens was undertaken; 10% of the 1314 patients admitted to BCRC between 9/72 and 1/77 had this organism recovered from their samples. The chief source of S. marcescens was found to be fresh uncooked foods and moist environmental areas. Advances in Staphylococcal phage typing made possible the detection of heavy shedding by mycosis fungoides patients colonized with S. aureus. Since then, isolation procedures are initiated when necessary.

Project Description:

This project is a continuing study of the microflora of cancer patients. This flora and its acquisition is determined by means of routine surveillance cultures which are taken at the time of patient admission and repeated twice each week on high-risk patients. The data thus generated is also used to investigate organism prevalence in the cancer patient population and to detect pathogens or unusual organisms. Such unusual organisms are studied extensively.

One such unusual organism was a coryneform bacterium that has been recovered from twenty patients, including four with bacteremia (one death) and three with infected lesions. The first two patients who had bacteremia had been exposed to a carrier who was found to be a shedder. These microorganisms are gram positive, non-motile organisms which have been shown by electron microscopy to contain metachromatic granules. Further characterizations of these organisms show that most strains utilize glucose and galactose, do not assimilate organic acids, contain m-DAP as the principal amino acid of the cell wall, have a G-C content of 64%, and divide by snapping. They are resistant to all antibiotics tested with the exception of vancomycin, occasionally rifampin, and rarely erythromycin. They most closely resemble coryneform bacteria in Group I of Yamada and Komagata, but differ in lack of utilization of organic acids. As they are often recovered from the skin of the groin, they most probably belong to that group of diphtheroids known commonly to colonize skin.

In a retrospective study, it was shown that 4,166 (17.4%) strains of Group D streptococci were recovered from 27,474 surveillance cultures taken from the nose, throat, gingiva, axilla, and rectum of the cancer patients. Additionally, 2,217 strains were recovered from 7,106 urine samples and 58 strains from 5,286 blood samples. Eleven percent of gingival cultures yielded Group D streptococci. Abscesses & lesions had recovery rates of 7.9 and 13.2%, respectively. Six of 11 premortem bloods which were positive for *Streptococcus faecalis* grew other organisms, chiefly gram negative bacilli. The seven *S. faecalis* recovered from postmortem bloods occurred together with gram negative bacilli or yeasts. *S. faecalis* was most prevalent, followed by *S. bovis*, *S. faecium*, *S. avium*, *S. equinus*, and *S. durans*. Although most strains were susceptible to penicillin as shown by MIC determinations, disc susceptibility tests gave results which placed them in the moderately susceptible range. Ampicillin susceptibility ranged from 71 to 93%. About half of *S. faecium* and *S. avium* strains were resistant to carbenicillin; the other species were more susceptible. Vancomycin, furadantin, and chloramphenicol had $\geq$ 82% susceptibilities to the Group D streptococci.

A review of 1,314 patients admitted to BCRC between 9/72 and 1/77 revealed that 104 (9.9%) had *Serratia marcescens* recovered from their cultures. Recovery rates based on the total number of surveillance cultures from each patient were low, approximately 2%. The highest rate was from the throat, 4%. There were 38 distinct serotypes among the 229 strains which were typed. The prevalent O antigenic type was O14 (115 strains), O4 (48 strains) and O1 (38 strains). These O antigenic types accounted for 84% of the isolates. The prevalent H antigens were 1, 4, 7, and 12. Among these antigens, H12 had the highest recovery rate; 39% of isolates had this antigen. H4 was next in frequency (19%), followed by H1 (12%) and H7 (9%). The most frequent serotypes were O14:H12 (36%), O4:H4 (10%), O1:H4 (6%), O1:H7 (5%), and O14:H10 and O4:H1 (both 4%). During the study, multiple isolates from the same patient yielded S. marcescens strains with the same O serotype, but with more than one H antigen. Further tests revealed that phase variation occasionally occurs among the Serratia (See Z01 CM 06254). Fresh uncooked foods,such as tomatoes and green peppers or orange juice, were sources of Serratia; less frequently they were found in or near moist environmental areas.

Phage typing of S. aureus allowed the tracing of S. aureus profusely shed by a patient with mycosis fungoides through clinic and hospitalized patient areas. Preventive measures, including isolation, contained the shed organisms and stopped their further spread. Since then, patients with this disease who carry S. aureus are isolated at admission.

It has been found that nasal cultures which yield filamentous fungi are often an indication of local or pulmonary infection with these organisms. Therefore, a procedure has been established to perform biweekly nasal fungal cultures on all hospitalized patients to determine their possible value in early detection of fungal infection.

Publications:

1. Young, V.M. and Morris, M.J. Antigenic structure, in The Genus Serratia, CRC Press, Inc., Cleveland, Ohio, In Press, 1978.

2. Young, V.M., Morris, M.J., Moody, M.R., Schimpff, S.C., and Wiernik, P.H., An analysis of Group D streptococci recovered from cancer patients, Infection, In Press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06223-09 COB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on Pseudomonas aeruginosa in a Cancer Research Center

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Others:	Viola Mae Young, Ph.D.	Head, Microbiology Section	CL NCI
	Maureen J. Morris	Laboratory Scientist	CL NCI
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COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Oncology

SECTION

Microbiology

INSTITUTE AND LOCATION

NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 1/6	1	1/6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The epidemiology of P. aeruginosa in cancer patients and the influence of various factors such as host immune response and antibiotic resistance on the pathogenicity of this bacterium in cancer patients are the prime objective of this study. Nonagglutinating antibodies have been demonstrated in the sera of patients who had been colonized and/or infected with P. aeruginosa. Protective capabilities of these antibodies are under investigation. Kinetics of nonagglutinating antibody response in cancer patients, burn patients, and cystic fibrosis patients immunized with pseudomonas vaccine are in progress.

An identification schema developed in our laboratory has been used to speciate approximately 1000 strains of the most frequently recovered Pseudomonas spp. other than P. aeruginosa. A schema for the identification of less common Pseudomonas spp. and Alcaligenes spp. is being evaluated.

PHS-6040
(Rev. 10-76)

Pseudomonas aeruginosa is frequently a causative agent of infection in cancer patients. Thus, the eradication and the prevention of such infections in this susceptible patient population remain essential. This study provides a continued monitoring of the incidence of *P. aeruginosa* in these patients. The epidemiology of this bacterium, its susceptibility to antibiotics, and the host immune response to it, are studied to elucidate the mechanisms by which *P. aeruginosa* can be controlled or eliminated. The prevalence and possible infectious role of other *Pseudomonas* spp. are also evaluated on an ongoing basis.

Serotypic identification of *P. aeruginosa* recovered from cancer patients and their hospital environs is performed on a routine basis. Studies of methods by which the serotyping of rough isolates can be facilitated are under investigation.

The epidemiological histories of *Pseudomonas* spp. other than *P. aeruginosa* have been expedited by the routine use of an identification schema developed in our laboratory for the saccharolytic species that are most frequently encountered. By now, this schema has been successfully used for approximately 1000 strains of these organisms. A schema for the identification of the less common nonsaccharolytic species and *Alcaligenes* spp. is under investigation.

Nonagglutinating immune pseudomonas antibodies have been shown to be effective in the protection of mice against *P. aeruginosa* infection. Nonagglutinating antibodies have also been demonstrated in the sera from fifteen cancer patients who had been colonized and/or infected with *P. aeruginosa* type 2. The protective capabilities of these specific nonagglutinating antibodies are under investigation. Studies of nonagglutinating antibodies in sera from cancer patients, burn patients, and cystic fibrosis patients who have been immunized with a pseudomonas vaccine are in progress.

Publications:

1. Moody, M.R., Kessel, R.W., Young, V.M., Fiset, P. The role of nonagglutinating antibody in the protracted immunity of vaccinated mice to *Pseudomonas aeruginosa* infection. Infection and Immunity, In Press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06254-09 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Research Activities Directed Toward Improvement of Microbiology Laboratory Techniques

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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TOTAL MANYEARS: 1 1/3	PROFESSIONAL: 1	OTHER: 1/3

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The evaluation of new methodology and its implementation, if proven more efficient or accurate, is the continuing objective of this project. A plate coagulase test which incorporates porcine plasma has proven to be a more sensitive method for detection of S. aureus carriers than the method previously utilized. Further studies to delineate the newly discovered phase variation of H antigens in Serratia marcescens allow more accurate serotyping of these organisms and thus is an aid in epidemiological investigations. Methods for determination of bactericidal and bacteriostatic activity of patient sera are being compared to determine optimal methods. A method of preventing overgrowth of yeasts in tissue cell lines inoculated with patient samples for recovery of viruses is under study. Thus far, Amphotericin B appears to be effective without being toxic to the tissue cell lines.

PHS-6040
(Rev. 10-76)

Project Description:

The objective of this project is to evaluate new methodology for the purpose of incorporating more efficient and accurate systems into the methods for recovery and identification of microorganisms from samples of cancer patients.

As *Staphylococcus aureus* is one of the major pathogens which cause infection in cancer patients, a plate coagulase test, which is more efficient for the identification of *S. aureus* than the usual laboratory procedures, has been under investigation. This test uses porcine plasma with Tween 80 in trypticase soy agar as a plate for direct sample inoculation and coagulase producing staphylococci produce a halo around the colonies. After testing over 100 samples by this method to prove its accuracy, it is now utilized to screen patients for *S. aureus* who have been placed on the Rifampin-Cloxacillin protocol to eradicate *S. aureus* (BCRC protocol #7314).

Further studies on *Serratia marcescens* to more accurately delineate their H antigens were performed. Two hundred and fifty isolates of *S. marcescens* from 90 patients were serotyped. Immobilization studies for the confirmation of H antigen identification demonstrated that a second H antigen was present in some cultures. When all of the H immobilization tests were complete, 21 of the 250 strains (8%) had demonstrable phase variation (a second phase that could be reversed to the first phase); 19 of these were diphasic and two were triphasic. H1 occurred in over half of the phase variants and was also a constituent phase of the two triphasic strains. H4 occurred in a little less than half of the phase variants and was an alternate expression with H1 in four strains. The knowledge that phase variation is present in the genus *Serratia* and that variability in H antigen was due to this phenomena, should be an aid to epidemiological investigations. This study represents the first report of phase variation within the genus *Serratia*.

Information as to the bacteriostatic and bactericidal activity of the sera from patients who are infected against the specific microorganism that causes the infection is often useful in selection of therapy. In a current study, a comparison is being made of bactericidal and bacteriostatic tests performed in tryptic soy broth or in 50% Mueller Hinton broth that has been adjusted to contain 75 mg of Ca++ and 30 mg of Mg++ per liter and 50% human serum. The 20 sera tested thus far have not given over a two-fold difference in titers between the two methods. While the Mueller-Hinton medium gives good results in the cidal tests, the bacteriostatic tests are difficult to read in this medium.

As overgrowth with yeasts create contamination problems in tissue lines that have been inoculated with samples for growth of viruses, a study has been initiated to inoculate duplicate cell lines, one of which has Amphotericin B added to inhibit the growth of yeasts. Thus far, half of the samples were contaminated by yeasts and all of the yeasts were inhibited in those cultures to which Amphotericin B had been added. Furthermore, this drug had no apparent effect on the tissue cells.

It is anticipated that these and other evaluations of new rapid and/or automated techniques will be undertaken during the next year.

Publications:

1. Morris, M.J., Young, V.M., Moody, M.R., Evaluation of a multitest system for identification of saccharolytic pseudomonads. J. Clin. Path. 69:41-47, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06938-04 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Environmental Studies to Prevent Hospital Acquisition of Potentially Dangerous Microflora

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)
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LAB/BRANCH
Clinical Oncology

SECTION
Microbiology

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NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 1/6	1	1/6

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Monitoring of the patients environment, and the personnel and materials with which they come in contact, is the purpose of this continuing project. Examples of activities for this project during the past year were: 1) use of an improved medium for detection of Staphylococcus aureus carriers; 2) evaluation of a new product for skin decontamination; 3) monitoring of air for fungal spores and detection of curtains as a heavily contaminated source of fungal spores, 4) detection of unsatisfactory decontamination procedures for isolation rooms that have been used by heavy shedders of pathogens, 5) a comparison of methods for sink drain decontamination, 6) continued surveillance of protective environment rooms, and 7) surveillance of hotel rooms which house cancer patients undergoing therapy on an out-patient basis. In addition, cultures of the patients' physical environment within the hospital are performed on a routine basis and reviewed for sources of contamination.

PHS-6040
(Rev. 10-76)

Project Description: Z01 CM 06938-04 COB

The purpose of this continuing project is to monitor the cancer patients' physical environment; the air, food and water, and the products used in their care in order to detect potentially pathogenic microorganisms. Personnel responsible for patient care are also cultured regularly to detect possible carriers. The use of the trypticase soy agar - porcine plasma medium (TSAM) (Project No. 06254) has been incorporated in our bi-annual employee survey of hands and nose for S. aureus. Comparison of this medium with the usual procedures for the detection of S. aureus carriers revealed that the TSAM detected a 20% carrier rate whereas the former procedure detected only a 13% rate.

Procedures utilized for decontamination of isolation rooms that had been used by heavy shedders of potentially pathogenic microorganisms were shown not to be fully effective. A study has been initiated to evaluate newer methods for the elimination of pathogens shed by such patients.

The use of a new product, Chlorhexidine gluconate (Hibiclens-Stuart Pharmaceuticals), for staff hand cleansing and patient baths is being evaluated for its effectiveness and compared to the provodine-iodine and "Phisohex" procedures.

As the fire-proofing material on the overhead beams had contained very numerous fungal spores that appeared to contribute to patient infection, decontamination with copper-8-quinolinolate had been undertaken. A continuing series of air samples using special fungal media were then initiated to monitor the ambient air. These cultures have revealed very few fungi. However, the incidence increases with unusual circumstances. Monitoring of the air during hospital maintenance and remodelling, during plumbing and electrical work which involves the ceiling has led to better planning for patient relocation during such procedures. In addition, it was found that the curtains in certain rooms were heavily contaminated with fungal spores, including Aspergillus spp. This possible source of contamination has been eliminated.

Cooked foods are the only diet given to high risk patients, as they are seldom contaminated. However, cultures of menu items are done on a scheduled basis in order to maintain high standards. All new items added to the menus are evaluated. Staff education seminars are conducted on the proper storage, preparation, handling, and serving of meals to increase awareness of the importance of proper procedures.

Various genera of bacteria; i.e., Pseudomonas, Klebsiella, Enterobacter, etc. are frequently recovered from the sink drains in patients' rooms. Not infrequently these strains are resistant to a variety of antibiotics. Two methods for the decontamination of sink drains are being evaluated to determine their efficacy. One is an electric coil-wrapped drain which can be heated to a temperature designed to eliminate bacteria through drying of the pipes on inner surface. This system was found to be ineffective at reducing numbers of bacteria present. The second was a valve cut-off system which allows disinfectant to be added to the drain and remain in contact with the contaminated surface a sufficient time for disinfection to occur. This system has proven effective, but re-contamination rates, and the effectiveness during practical use remains to be determined.

Cancer patients undergoing therapy on an out-patient basis are housed in a near-by hotel. During the past year, the facility used was changed from a motel to a hotel which necessitated a visual inspection and periodic sampling of the patient rooms and facilities to ensure that their environment was pathogen free. Thus far, although suggestions have been made to reduce the numbers of fungal spores, the new facility has proven to be free of potentially dangerous microflora.

Controlled environments designed to protect patients from hospital-acquired microorganisms, continue to be evaluated. An analysis of microbiologic data revealed that results of cultures taken at 5 sites within the protective room provided most of the information necessary for proper maintenance of the pathogen-free environment at a considerable savings in time and cost.

Publications:

1. Schimpff, S.C., Hahn, D.M., Brouillet, M.D., Young, V.M., Fortner, C.L., and Wiernik, P.H. Infection prevention in acute leukemia: comparison of basic infection prevention techniques with standard room reverse isolation with reverse isolation plus added air filtration. Leukemia Res. (In Press), 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06972-01 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Antibiotic Studies: Evaluation of New Drugs and Their Characteristics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Maureen J. Morris	Laboratory Scientist	CL NCI
	Stephen C. Schimpff, M.D.	Head, Infections Research	CL NCI

COOPERATING UNITS (if any)
Hazleton Laboratories, Vienna, Va.

LAB/BRANCH
Clinical Oncology

SECTION
Microbiology

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NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	3/4	1/4

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

"Normal" microflora can cause serious infections in cancer patients and preventive measures by which these infections can be eliminated are essential. The objective of this study is to evaluate the effectiveness of new antimicrobial agents against such microflora. Enterobacteria and S. aureus were susceptible to netilmicin, tobramycin, gentamicin, and amikacin. Susceptibility of P. aeruginosa was variable. S. faecalis was susceptible to netilmicin and resistant to amikacin. Both ticarcillin (T) and carbenicillin (C) were effective against gram positive bacteria, E. coli, and P. mirabilis, but T was the more active against P. aeruginosa than C. Piperacillin, azlocillin, and mezlocillin were more active than C and T against most genera. Daunorubicin and cytosine arabinoside exerted an antagonistic effect on the activity of the aminoglycosides mentioned above, but not against kanamycin. Two new cytotoxic agents, Peptochemio and Ledacrine, had no antifungal activity, but miconazole (M) and miconazole nitrate (MN) were inhibitory against C. albicans and the majority of T. glabrata strains. Rifampin frequently enhanced this inhibition. The antifungal R41400 did not effect most T. glabrata and Candida spp. other than C. albicans.

Microorganisms that are considered "normal flora" when harbored by the uncompromised host often cause serious infections in the compromised cancer patient and means by which these infections can be eliminated or prevented are essential to the supportive therapy of this susceptible patient population. Newer antimicrobial agents that have activity against a broad spectrum of microorganisms are under continual development and many of these agents have been recommended for use in the suppression of such microorganisms as E. coli, K. pneumoniae, P. aeruginosa, S. aureus, C. albicans, etc. It has been recommended that these antimicrobial agents be used singly or in combination with each other. This study is designed to obtain these new agents as they become available and to evaluate their effectiveness against these infectious microorganisms (See Project 06266-08).

The combination of aminoglycosides and penicillin derivatives have been shown to be synergistic against P. aeruginosa as well as other gram negative bacilli. The susceptibilities of bacterial strains more frequently recovered from patients at the BCRC to four aminoglycosides and two semisynthetic penicillins were determined. Netilmicin, tobramycin, gentamicin, and amikacin inhibited 90% or more of the enterobacteria strains; E. coli, P. mirabilis, and K. pneumoniae, at concentrations in susceptible ranges. Conversely,only 47 to 57% of P. aeruginosa strains were inhibited by the first three antibiotics at the same concentrations and only 75% of the strains was susceptible to amikacin. Thirty-seven percent was resistant to more than 24 µg/ml of tobramycin or gentamicin, whereas only 13% was resistant to the same concentration of netilmicin and 8% was resistant to more than 40 µg/ml of amikacin. All strains of S. aureus were susceptible to amikacin and netilmicin and 88% were susceptible to gentamicin and tobramycin. Among S. faecalis strains, 93% was susceptible to netilmicin but none was to amikacin. Susceptibilities to gentamicin and tobramycin were 26% and 23%, respectively. Ticarcillin and carbenicillin inhibited 100% of P. mirabilis strains, S. faecalis strains, and S. aureus strains as well as 88% of E. coli strains. The former antibiotic was more effective against P. aeruginosa (89% susceptible) than was the latter agent (77%). Only 32% and 8% of the K. pneumoniae strains were susceptible to ticarcillin and carbenicillin, respectively.

As there had been no recommendations of ticarcillin zone size interpretations for K. pneumoniae when tested by disc susceptibility tests, most laboratories utilized the zone size parameters recommended for E. coli and P. mirabilis. A comparative review of ticarcillin zone sizes using a 75 µg disc and MIC's for this organism revealed a discrepancy between the two test methods. To study this variation, the susceptibilities of 150 K. pneumoniae strains to ticarcillin and carbenicillin by disc and MIC methods were determined. Carbenicillin disc and MIC results correlated well as did MIC results of carbenicillin and ticarcillin for a particular organism. However, over half of the strains gave disc zone size results that did not correlate with the MIC's; i.e., susceptible by disc but resistant by MIC. The majority of these strains had zone sizes which were within 2 mm of the intermediate zone sizes recommended for the other bacteria. Also, some strains that had MIC's in the resistant range had zone sizes well within the "susceptible" zone range (up to 19 mm). It was concluded that, for the present, the carbenicillin disc gives a more valid indication of ticarcillin susceptibility or resistance than does the 75 µg ticarcillin disc provided by the manufacturer.

The antimicrobial activities of three newer semisynthetic penicillins; azlocillin, mezlocillin, and piperacillin was compared with those of ticarcillin and carbenicillin. Various bacteria; e.g., *E. coli*, *K. pneumoniae*, *Serratia* spp., *P. mirabilis*, *P. aeruginosa*, Group D streptococci, *S. aureus*, that had been freshly isolated from cancer patients were used as test organisms. Piperacillin, azlocillin, and mezlocillin had the best range of activity as $\geq$ 90% of all species, except *S. marcescens*, were inhibited by concentrations $\leq$ 25 µg/ml; 89% of *S. marcescens* was susceptible to 100 µg/ml. Piperacillin and azlocillin had excellent activity against *P. aeruginosa*, 92% and 88%, respectively. The newer penicillins were highly active against the test organisms and had the added advantage over ticarcillin and carbenicillin of effectiveness against *K. pneumoniae*.

Daunorubicin and cytosine arabinoside were tested individually and in combination with each of five aminoglycosides; netilmicin, tobramycin, amikacin, gentamicin, and kanamycin, to determine their effects on strains of *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. At clinically achievable plasma levels, neither of the cytotoxic agents had antimicrobial activity, but both exerted antagonistic effects on the antimicrobial activities of gentamicin, tobramycin, netilmicin, and amikacin, but not on the activity of kanamycin. Gentamicin was most affected as MIC's were increased with DNR and AraC in 13 of 15 *K. pneumoniae* strains and in 8 of the 15 *P. aeruginosa* strains. *E. coli* MIC's were unchanged.

Two new cancer chemotherapeutic agents, Peptochemio and Ledacrine, were tested for activity against *Candida* spp. and *T. glabrata*. No activity was demonstrated when tested individually or in combination with 5 µg rifampin, at concentrations from 0.25 µg/ml to 128 µg/ml.

Over 400 fresh clinical isolates of yeast from cancer patients were tested by an in vitro agar dilution method against miconazole (M), miconazole nitrate (MN), and R41400, a miconazole derivative, individually and in combination with 5 µg of rifampin. Most strains of *C. albicans* were susceptible to $\leq$ 0.5 µg/ml of all miconazoles. Higher concentrations of M and MN were required to inhibit other *Candida* spp; they were resistant to R41400 as were all *T. glabrata* strains. 75% and 88% of the latter strains were inhibited by 1 µg/ml of M and MN, respectively. The rifampin and MN combination was most frequently synergistic against *C. albicans* whereas the R and M combination was most effective against *T. glabrata*; many M resistant strains of *T. glabrata* became susceptible upon addition of R. Synergy between rifampin (R) and R41400 was not observed.

Publications:

1. Hahn, D.M., Schimpff, S.C., Fortner, C.L., Smyth, A.C., Young, V.M. and Wiernik, P.H. Infection in acute leukemia patients receiving oral nonabsorbable antibiotics. Antimicrob. Ag. Chemother. (In Press) 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06043-07 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Automated Monitoring of Drug Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	.75	1.75

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to provide an efficient and rapid method of automated collecting of data concerning the clinical trials of experimental drugs.

The system of computer processing of drug administration information provides accurate periodic summaries of all BCRC patient's drug therapy. Each drug card is typed containing the name of the drug, dose, route of administration, frequency, date, and patients name and medical record number. Each card also contains the direct administration record as charted by the nurses at the time of administration. These cards are processed by optical character recognition and produce a weekly summary of all drugs ordered and their administration record, organized by patient and by problem oriented system.

This system fulfills all the requirements of drug ordering, administration and recording in addition to providing machine-readable data for computer input. This system eliminates the transcription operations usually associated with preparation of input for computers, and provides a data pool for clinical studies.

PHS-6040
(Rev. 10-76)

Project Description: Z01 CM 06043-07 COB

The objective of this project is to provide an efficient and rapid method of collecting data concerning the clinical trials of experimental drugs.

This is being studied by a computerized method of ordering and recording all drug therapy.

A system of processing drug administration information provides accurate, weekly summaries of each patient's complete drug therapy. The physician writes each drug order directly on a medication card, producing three copies: the pharmacy copy (for entry to a patient drug profile), a copy for medical records and a copy for automated analysis. The nurse charts medications given directly on the first copy of the drug form at the time of drug administration. This data is read weekly by optical character recognition. The data is collated and patient drug summaries are printed listing drug, dose, total dose, frequency of administration and a daily record of administration or omission of each drug. A monthly drug utilization report is also obtained as a by-product.

The problem number and statement according to the Weed's problem oriented health record system has been incorporated into the form which allows each drug to be listed by problem number on the weekly drug summary for each patient. Space on the drug form has also been allotted for the patient's height, weight, and body surface area. This gives the pharmacist enough data to recalculate all drug doses listed in research protocols. Not only does this recalculation protect the patient, but it also protects the research environment by insuring the accuracy of all doses administered. This system fulfills all the requirements of drug ordering, administration and recording, in addition to providing machine-readable data for computer input. The summaries are checked by the pharmacists, and become a permanent record of drug therapy both in the patient's chart and in the pharmacy. This system eliminates the transcription operations usually associated with preparation of input for computers, and provides a data pool for clinical studies, analyzing drug effect, toxicity, and drug interaction. This system has reduced errors in drug administration and has increased staff awareness of the problems inherent in quality drug administration. A complete list of all drugs administered to the patient are recorded by problem numbers in a concise and orderly fashion in the patient chart. This system, as outlined, has provided a more comprehensive and efficient record of cancer chemotherapy drug trials.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06236-07 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Role of the Pharmacist in Obtaining a Patient's Drug and Allergy History

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Head, Clinical Research Pharmacy Service, COB-NCI

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John F. Bender, Clinical Pharmacist, COB-NCI

COOPERATING UNITS (if any)

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TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to evaluate the feasibility of having a pharmacist interview all patients referred to the BCRC to obtain a complete drug and allergy history.

Three major areas of medication history are investigated: prescription medications, over-the-counter (OTC) medications, and previous allergic reactions. The original copy of the interview report is placed in the history section of the patient's hospital chart. Duplicate copies are retained in the patient's drug profiles in the pharmacy. The pharmacist is responsible for placing all known allergic reaction information on the problem sheet of the patient's chart. All medications brought to the hospital by the patient are obtained at the time of the interview, and are stored in the pharmacy during the period of hospitalization.

In addition, new outpatients to BCRC are also interviewed by the pharmacist as they receive their first outpatient prescriptions.

These interviews have provided the pharmacist with patient contact at the time of admission which has increased the pharmacists awareness of patient care.

These interviews have yielded information useful in the care of the patient.

Project Description

The purpose of this study is to evaluate the feasibility of having a pharmacist interview all patients referred to the BCRC to obtain a complete drug and allergy history.

A guideline for the interview has been developed which investigates three major areas: prescription drugs, over-the-counter (OTC) drugs and allergy/sensitivity history. All pertinent information, particularly regarding allergy history, is brought to the direct attention of the physician. The pharmacist is also responsible for placing all known allergic reaction information on the problem sheet of the patient's chart and on the front of the patient's Kardex. The information obtained from the interview is placed in the history section of the patients chart. Duplicate copies are retained in the patient's drug profiles in the pharmacy.

All medications brought to the hospital by the patient are obtained at the time of the interview, and are stored in the pharmacy during the period of hospitalization.

During the last year, the outpatient pharmacist has begun obtaining the same drug and allergy history information on new outpatients to BCRC, at the time they receive their initial prescriptions.

These interviews have yielded information not obtained by other members of the health care team which has been of patient care value.

The drug and allergy interview by the pharmacist has become an integral part of the clinical program. The present study will be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06237-08 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Development of a Clinical Research Pharmacy Service

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Head, Clinical Research Pharmacy Service, COB-NCI

OTHER: William R. Grove, Assistant Director, Clinical Research Pharmacy Service, COB-NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☑ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to determine the role of the pharmacist in the patient care area, and as a member of the health care team actively contributing to the patient's care and research efforts.

The pharmacist has proven that he can be patient-oriented rather than product-oriented. The pharmacist utilizing new methods has become a valuable member of the research health care team and in contributing directly to the patient care. The following has been accomplished: improved chemotherapy records, monitored chemotherapy orders against protocols, improving drug prescribing practices by promoting the rational and safe use of drugs, preventing potential drug-drug interactions, detecting and preventing adverse drug reaction, detecting drug induced laboratory test abnormalities, detecting possible drug induced disease, providing drug information, continuing education for various members of the health care team, counseling the patient on proper use of drugs, and detecting and preventing intravenous admixture incompatibilities.

PHS-6040
(Rev. 10-76)

The objective of study is to determine the role of pharmacist in a research/patient care area.

The pharmacist is located in the midst of the patient care area in order to allow him to investigate new concepts, approaches, and research opportunities to aid in the patient care. The pharmacist attends morning and evening ward rounds, reviews charts, interviews patients and is familiar with the patients' condition. Pharmacy rounds are convened immediately after the morning medical rounds. All pharmacists attend, as all new problems are reviewed. This provides the pharmacists with all of the patient current problems. Therefore he can intelligently discuss therapy problems with the patients's physician during the working day. With this information, he can also provide the staff with unsolicited information.

An integral part of this service is the therapy surveillance system. The nucleus of the drug surveillance system is the individual patient drug profile and investigational drug record. The pharmacist receives a direct copy of the physician drug order and transcribes it to the patient drug profile and chemotherapy record. The drug record includes the patient name, primary and secondary problems, physician name, known allergies or idiosyncrasies and all drug orders. The pharmacist reviews each new drug order for completeness and recalculates all protocol drug dosages then reviews the drug profile against the new order for drug interactions, laboratory problems, dietary problems and possible adverse drug reactions. Problems are brought to the physicians attention. The drugs are then dispensed through a unit dose system which provides accurate records. All returned doses to the pharmacy are investigated for reason of non-compliance. The monitoring system involves evaluating the necessity of continued medication orders.

The pharmacist has shown that he can be patient oriented rather than product oriented. The pharmacist utilizing the above methods has become a valuable member of the research health care team and contributes directly to the patient care. The following has been accomplished: improved chemotherapy records, monitored chemotherapy orders against protocols, improving drug prescribing practices by promoting the safe and rational use of drugs, preventing potential drug-drug interactions, detecting and preventing adverse drug reactions, detecting drug induced laboratory test abnormalities, detecting possible drug-induced diseases, providing drug information, counseling the patient on proper use of drugs, and detecting and preventing intravenous admixture incompatibilities. This has reduced drug exposure to the patient, decreased nursing and pharmacy time spent administering and preparing drugs and reduced cost.

A higher quality of drug prescribing and reduced medication errors has contributed toward better research/patient care and has enhanced the quality of the research data.

Drug information is becoming a major by-product of locating the pharmacist in the patient care area. This information is being requested by all members of the health care team. Continuing education is also involved in this process of providing drug information. The impact of this service will continue to be studied by the audit process.

The pharmacist is making rounds with a nurse to improve compliance of patient taking non-absorbable oral antibiotics and other medications. A pharmacist is doing audiograms of patients receiving potentially ototoxic agents.

Present studies will be continued and additional methods to improve patient care, will be further researched.

PUBLICATIONS:

Bender,J., Grove,W., Fortner,C.L.; High-Dose Methotrexate with Folinic Acid Rescue; J. Am. Soc. Pharm. 34: 961-965, 1977.

Hahn,D., Schimpff,S., Young,V., Fortner,C., Standiford,H., Wiernik,P.; Amikacin and Cephalothin: An Empiric Regimen For Granulocytopenia Cancer Patients; Antimicr Ag Chemother 12, No. 5, p. 618-624, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06912-04 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Antineoplastic Drug Displays for the Computerized Problem-Oriented Medical Information System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: William R. Grove, Assistant Director, Clinical Research Pharmacy Service, COB-NCI
Clarence L. Fortner, Head, Clinical Research Pharm. Service, COB-NCI

OTHER: Paul Chang, M.D., Senior Investigator, Med. Oncology Service, COB-NCI
Robert Esterhay, M.D., Senior Investigator, Med. Oncology Service, COB-NCI
Peter Walton, M.D., PROMIS, Laboratory, Dept. Med. University of Vermont Burlington, Vermont
Peter Wiernik, M.D., Acting Director, BCRC, COB-NCI

COOPERATING UNITS (if any)

PROMIS Laboratory, Dept. of Medicine, University of Vermont

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.6	.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to prepare referenced summarized pharmacologic information which is readily retrievable at the touch-sensitive cathode ray screen terminal located at the BCRC nursing station.

Current literature is reviewed to obtain accurate and complete drug information on antineoplastic agents. The standardized format consists of separate display pages for information as follows: DRUG NAME, CHECK PROBLEM LIST FOR, SIDE EFFECTS TO WATCH FOR, DRUG INTERACTIONS, TEST INTERFERENCES, USUAL DOSAGE, PHARMACEUTICAL DATA, MECHANISM OF ACTION, AND METABOLISM AND EXCRETION. Reference codes are included with all pages of information, which may be further explored by contacting either the Clinical Research Pharmacy Service of the BCRC or the PROMIS Laboratory, University of Vermont. After completion, display sequences are sent to the PROMIS laboratory to be audited for accuracy, completeness, and correctness of clinical judgement before being entered into the computerized system.

The completed antineoplastic drug displays provide ready access to referenced summaries of the current medical literature. They form an integral part of the contribution to improved patient care and improved medical records played by the PROMIS system.

PHS-6040
(Rev. 10-76)

Project Description: Z01 CM 06912-04 COB

The objectives of this project are to prepare referenced summarized pharmacologic information which is readily retrievable at the touch-sensitive cathode ray screen terminal located at the BCRC nursing station. These displays are to be complete, concise and accurate summaries of currently-available literature. Additional antineoplastic drugs are added to the computerized drug displays already on file with the PROMIS system, Burlington, Vermont.

Current literature is reviewed to obtain accurate and complete drug information on antineoplastic agents. This information is then organized to a specific format to conform with the over 20,000 displays currently included in the PROMIS system. The standardized format consists of separate display pages for information as follows: DRUG NAME, CHECK PROBLEM LIST FOR, SIDE EFFECTS TO WATCH FOR, DRUG INTERACTIONS, TEST INTERFERENCES, USUAL DOSAGE, PHARMACEUTICAL DATA, MECHANISM OF ACTION, AND METABOLISM AND EXCRETION. Reference codes are included with all pages of information, which may be further explored by contacting either the Clinical Research Pharmacy Service of the BCRC or the PROMIS Laboratory, University of Vermont. After completion, display sequences are sent to the PROMIS Laboratory to be audited for accuracy, completeness, and correctness of clinical judgement before being entered into the computerized system. Previously entered data may be updated as necessary by providing the new information and references to the PROMIS laboratory for incorporation.

The completed antineoplastic drug displays provide ready access to referenced summaries of the current medical literature. They form an integral part of the contribution to improved patient care and improved medical records played by the PROMIS SYSTEM.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06937-03 COB

PERIOD COVERED
OCTOBER 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

A Patient Oriented Outpatient Drug Distribution Service

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Director, Clinical Research Pharmacy Service, COB-NCI

OTHER: William R. Grove, Clinical Pharmacist, COB-NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS: 1.7	PROFESSIONAL: 1.7	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to provide optimal patient care through the pharmacist's use and maintenance of an individual outpatient drug profile to provide necessary drug information and administration instructions to outpatients, and to dispense prescriptions to outpatients.

When the pharmacist receives the prescriptions from an outpatient, he reviews them for completeness and against any previously prescribed drugs for drug-drug interactions and any known allergies. This new prescription information is then added to the outpatient drug profile. When a patient is admitted or discharged, the outpatient profile serves as a method by which the pharmacist may verify that necessary medications are continued. The prescriptions will then be prepared and dispensed, with the pharmacist advising the patient of common side effects and specific administration instructions. Patient compliance is stressed by the pharmacist.

PHS-6040
(Rev. 10-76)

The objectives of this project are to provide optimal patient care through the pharmacist's use and maintenance of an individual outpatient drug profile, to provide necessary drug information and administration instructions to outpatients, and to dispense prescriptions to outpatients. These studies will include a method of best detecting drug-drug interactions, drug-food interactions and known patient allergies prior to dispensing the drug to the patient. Also the most appropriate and efficient method of utilizing the outpatient drug profile is to be determined.

New patients will be interviewed by the pharmacist to determine prior drug usage and any known allergies or sensitivities. Any relevant information found is included in the appropriate area of the outpatient drug profile as well as brought to the attention of the physician.

When a prescription is received by the pharmacist, he reviews it for completeness, rational drug therapy, and against the outpatient drug profile for drug-drug interactions and allergy information. Any changes in therapy particularly regarding antineoplastic therapy, are verified with the physician. Several inadvertant changes with potentially serious consequences have been averted by this method. In addition, any situations where prescriptions are not written that should have been, particularly with chronic medications, are investigated.

When a patient is admitted or discharged, the outpatient drug profile serves as a method by which the pharmacist may verify that necessary medications are continued.

The outpatient profile is useful in providing answers to questions from physicians concerning previous outpatient medications.

The patient will be advised by the pharmacist, at the time of dispensing, of common side effects, drug-drug interactions, drug-food interactions, and specific administration instructions, to ensure optimal effectiveness from the medications. Reference standards for patient consultation are being prepared by the pharmacist, particularly in the area of antineoplastic agents. The importance of patient compliance is stressed by the pharmacist. When follow-up instructions indicate poor patient compliance, the pharmacist promptly notifies the physician.

Results indicate proper use of this individual outpatient profile increases proper utilization of outpatient prescriptions and increased patient compliance.

SMITHSONIAN SCIENCE I TION EXCHANGE PROJECT NUMBER (Do NOT this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06238-07 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Development of a Unit Dose Service and the Preparation of Parenteral Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Head, Clinical Research Pharmacy Service, COB-NCI

OTHER: William R. Grove, Clinical Pharmacist, COB-NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	2

CHECK APPROPRIATE BOX(ES)

☑ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To improve patient care through a new drug delivery system and to provide more accurate information regarding the disposition of drugs.

The two systems, unit-dose and I.V. admixture, have proven to be advantageous over previous drug distribution systems. They have placed the drug preparation and distribution in the hands of the pharmacist. These systems have accomplished the following: reduced medication errors, reduced medication cost, enhanced the pharmacist as a drug information consultant by relating him more closely to the patient, reduced the possibility of contamination of the drug, permits nurses to use time more effectively in direct patient care, and in general allows the hospital to demonstrate control over the distribution of all drugs. In addition, the pharmacist has instituted record systems that have improved retrieval of the patient's drug records.

-6040
(Rev. 10-76)

Project Description: Z01 CM 06238-07 COB

The objective is to improve patient care through a new drug delivery system and to provide more accurate information regarding the disposition of drugs.

A unit dose of medicine is a single dose of medicine packaged for an individual patient. This single dose package is labeled with a name, strength, and control number of the drug, and it is ready to be administered to the patient. These drugs are ordered by the physician and they are placed in the individual patients drawer of a medication cart by the pharmacist. This cart is designed so that there is a single drawer for each patient's drug. All of the parenteral products are prepared aseptically in a laminar air flow enviroment, and are dispensed in a clearly labeled ready to use form. Parenteral products are prepared for not more than a 24 hour period to ensure full potency. The parenteral drugs are not placed in this cart. They are carried directly to a refrigerator in the nursing unit. Control procedures for checking the composition of the product prepared, and for monitoring bacterial and particulate matter contamination of these preparations have been instituted. Pertinent information regarding the drugs dispensed is recorded on specially designed forms that are filed according to the patient's name. This information is also recorded by drug and lot number for each investigational agent.

The two systems, unit-dose and I.V. admixture, have proven to be advantageous over the old ward stock system of drug distribution. They have placed the preparation and distribution in the hands of the pharmacist. These systems have accomplished the following: reduced medication errors, reduced medication cost, enhanced the pharmacist as a drug information consultant by relating him more closely to the patient, reduced the possibility of contamination of the drug, permits nurses to use time more effectively in direct control over the distribution of all drugs. In addition, the pharmacist has instituted record systems that have improved retrieval of the patient's drug record. Physicians utilized these records daily in ordering medications for patients and have found them to be a useful and accurate source of information.

During the past 12 months the pharmacy service has dispensed approximately 229,763 oral doses of medication to inpatients, 39,701 I.V. admixtures, 11,618 doses of parenteral investigational cancer chemotherapeutic agents, and 3,838 sundry sterile preparations.

By reducing medication errors , and improving the accuracy of the records of disposition of cancer chemotherapeutic agents and the other unit dose drugs the quality of patients care has increased. These delivery systems have been integrated with the development of the Clinical Research Pharmacy Service which is providing the patients with better care. Thus, the research drug data will be improved.

SMITHSONIAN SCIENCE [illegible] EXCHANGE
PROJECT NUMBER (Do NOT [illegible] this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06943-03 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Audit of the Outpatient Drug Profile

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: William R. Grove, Assistant Director, Clinical Research Pharmacy Service, COB-NCI

OTHER: Leighton C. Burks, Clinical Pharmacist, COB-NCI
Clarence L. Fortner, Head, Clinical Research Pharmacy Service, COB-NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS: .6
PROFESSIONAL: .6
OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to promote optimal patient care through appropriate utilization of the outpatient drug profile. The process audited is the pharmacist's completion and utilization of the outpatient drug profile. The audit criteria previously defined as audit standard, method, interval, audit form, and feedback system were used.

A third audit of fifteen randomly selected outpatient profiles was performed. Several observations were made, including: Prescription entries in the profile are being completed appropriately in 93% of the instances; no significant drug-drug interactions were dispensed; no drugs were dispensed in violation of any known allergies or sensitivities; all prescriptions written and filled were within the boundaries of rational drug therapy; 100% of drugs written on research protocols were recorded in the appropriate log records; 83% of the prescriptions were dispensed with an indication if a consultation was performed by the pharmacist; problem numbers were indicated on 68% of prescriptions written.

Several recommendations were made to the Clinical Research Pharmacy Service staff, based upon the results of this audit, to improve the utilization of the outpatient drug profile.

PHS-6040
(Rev. 10-76)

The purpose of this study is to promote optimal patient care through appropriate utilization of the outpatient drug profile.

Outpatient drug profiles are randomly selected and audited against the previously established audit criteria, including audit standard, audit method, audit interval, audit form, and feedback system. Each profile prescription entry is evaluated and analyzed against the audit criteria for completeness, rational drug therapy and research protocols.

A third audit of fifteen randomly selected outpatient profiles was performed. Several observations were made, including: prescription entries in the profile are being completed appropriately in 93% of the instances; no significant drug-drug interactions were dispensed; no drugs were dispensed in violation of any known allergies or sensitivities; all prescriptions written and filled were within the boundaries of rational drug therapy; 100% of drugs written on research protocols were recorded in the appropriate log records; 83% of the prescriptions were dispensed with an indication if a consultation was performed by the pharmacist; problem numbers were indicated on 68% of prescriptions written.

The results of each audit are reviewed with the Clinical Research Pharmacy Service staff to discuss methods of improvement in the utilization of the outpatient drug profile to promote optimal patient care.

This study will continue with additional audits to be performed periodically. The results of subsequent audits will be compared against previous ones to determine if improvement has occurred.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM 06961-02 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Establishment of a Pharmacy Clinical Clerkship in Oncology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Head, Clinical Research Pharm. Service, COB-NCI

OTHER: William R. Grove, Clinical Pharmacist, COB-NCI
Robert Kerr, Pharm. D., Director, clinical Pharmacy, University of MD
John Bender, Clinical Pharmacist, COB-NCI
Peter H. Wiernik, M.D., Acting Director, BCRC, COB-NCI

COOPERATING UNITS (if any)

University of Maryland, School of Pharmacy

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☑ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The BCRC pharmacy has joined with the University of Maryland School of Pharmacy in establishing a clinical clerkship in oncology for students obtaining their Doctor of Pharmacy Degree. This clerkship is an elective four week rotation.

The objective is to provide a clinical experience in oncology. The students are to understand the criteria for oncology research/treatment protocols, i.e. patient selection, study design and evaluation of study. They will gain experience in cancer therapy, toxicities and the management of non-neoplastic concurrent medical/social problems.

The pharmacy student should gain experience in both the inpatient and ambulatory areas.

PHS-6040
(Rev. 10-76)

Project Description Z01 CM 06961-02 COB

The Clinical Research Pharmacy Service has joined with the University of Maryland School of Pharmacy in establishing a clinical clerkship in oncology for students obtaining their Doctor of Pharmacy Degree. This clerkship is an elective 4 week rotation.

The objectives are to provide a clinical experience in oncology. The students are to understand the criteria for oncology research/treatment protocols i.e., patient selection, study design and evaluation of study. They will gain experience in cancer therapy, toxicities and the management of non-neoplastic concurrent medical/social problems. They will learn the effective and reliable reference sources for the most current information on cancer chemotherapy.

The pharmacy student will gain experience in both the inpatient and ambulatory areas.

These students will be directed through the Clinical Research Pharmacy Service with support from the Medical Oncology Branch.

This new program has had only six rotations during the past year and will continue for further evaluation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06990-01 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Role of the Pharmacist in Monitoring Patients Receiving Ototoxic Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: John F. Bender, Clinical Pharmacist, COB-NCI

OTHER: Clarence L. Fortner, Head, Clinical Research Pharmacy Service, COB-NCI
Stephen Schimpff, M.D., Head, Infectious Research Section, COB-NCI
Peter H. Wiernik, Acting Director, BCRC, COB-NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☑ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to evaluate the feasibility of Pharmacist performed audiometric monitoring of patients who are receiving potentially ototoxic therapy.

Patients receiving potentially ototoxic drugs have baseline pure tone audiograms performed by the pharmacist utilizing a portable audiometer. A follow-up audiogram is obtained at the completion of therapy. All abnormal follow-up audiograms are noted and brought to the attention of the physician. Additional follow-up audiograms are scheduled in those patients with abnormal results, to determine the reversibility of the toxicity.

This function has allowed the pharmacist significant patient contact and a greatly increased awareness of the need for a multidisciplinary approach to patient care.

This function has also provided a significant opportunity for the patient to utilize the pharmacist as a source person concerning their drug therapy.

This study has demonstrated that the pharmacist can contribute significantly to the care of the patient by performing clinically relevant audiometric studies of patients at high risk of developing ototoxicity.

S-6040
(Rev. 10-76)

Project Description: Z01 CM 06990-01 COB

This study was designed to evaluate the feasibility of having a pharmacist conduct pure - tone audiometric studies on patients receiving potentially ototoxic drugs. All audiograms are conducted in the patients semi-private room utilizing a portable beltone audiometer (Model 10D).

Training of a pharmacist was accomplished in conjunction with the audiology department of the University of Maryland Hospital. This pharmacist in turn educated the other pharmacists in the proper technique of obtaining a pure-tone audiogram.

The pharmacist is responsible for obtaining baseline and follow-up audiograms on all patients randomly allocated to receive empiric antibiotic therapy which includes an aminoglycoside. Abnormal findings are related to the patients physicians.

The pharmacist has been able to demonstrate a 5% over all occurance of ototoxicity associated with aminoglycoside antibiotics.

This function has given the pharmacist a significant and productive amount of patient contact time and interaction with research protocols. Not only has this increased the pharmacists awareness of the need for a multidisciplinary approach to patient care and research data collection, but have increased their utilization of the pharmacist as a drug information source person.

This study demonstrates the ability of the pharmacist to contribute significantly to the care of the patient and the compilation of research data by conducting accurate audiometric studies of patients at high risk of developing ototoxicity. This study will continue as the pharmacist is now involved in the study of other potentially ototoxic agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06991-01 COB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Audit of the deHaen Drugs-in-Use Drug Information System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: William R. Grove, Assistant Director, Clinical Research Pharmacy Service, COB-NCI

OTHER: Clarence L. Fortner, Head, Clinical Research Pharmacy Service, COB-NCI

COOPERATING UNITS (if any)

Peter P. Lamy, Ph.D., School of Pharmacy, University of Maryland

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.75	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to audit the deHaen Drugs-In Use drug information system for accuracy and completeness of abstraction.

A random selection process was designed to select deHaen abstracts for auditing. The corresponding original reference is then obtained and completely reviewed to determine the accuracy and completeness of the abstraction.

An interim result of auditing 75 articles reveals an unexpectedly high error rate. Fifty-two percent of cards have been found to contain at least one error. Seventy percent of the errors detected involve the accuracy of the abstraction.

The study will continue until 100 deHaen abstracts have been audited. At that time the data will be analyzed to determine rate, type cause and significance of the errors observed. Recommendations will be made concerning the use the deHaen abstracts as a source of drug information.

The deHaen Drugs-In-Use drug information system provides the subscriber with abstracts of articles appearing in over 400 medical and scientific journals. As such, it is a widely-used source of drug information.

The objective of this project is to audit the deHaen Drugs-In-Use drug information system for accuracy and completeness of abstraction.

A random selection process was designed to select abstracts for auditing. The corresponding original reference is then obtained and completely reviewed to determine the accuracy and completeness of the abstraction.

A sample of 100 abstracts will be audited. For the purposes of this study, this will provide a maximum tolerable error in the study of 10%, with 95% confidence.

An interim result of auditing 75 articles reveals an unexpectedly high error rate. Fifty-two percent of cards have been found to contain at least one error. Seventy percent of the errors detected involve the accuracy of the abstraction.

SUMMARY
Laboratory of Clinical Biochemistry
October 1, 1977 to September 30, 1978

Research programs, the technical capabilities, and the administrative organization of the Laboratory of Clinical Biochemistry have evolved during the past year to yield a dynamic research group with exciting and important research goals.

In biochemical pharmacology, studies establish that quinone and heterocyclic quinone anticancer agents such as adriamycin, daunorubicin, streptonigrin, mitomycin C, and lapachol, are catalytically activated to a free radical state by a microsomal system requiring NADPH. We determined that a microsomal enzyme catalyzing this transition was NADPH cytochrome P450 reductase. In addition to the quinone antibiotics, we determined that actinomycin D, a quinoid structure, is metabolized to a free radical with secondary formation of superoxide. Microsomes and NADPH cytochrome P450 reductase both catalyze the actinomycin D facilitated oxygen consumption.

From these studies we have proposed that activated free radical-drug intermediates may be site-specific free radicals which associate selectively and preferentially with macromolecular structures causing cell damage and subsequent cell cytotoxicity.

We expanded our research on the aldo-keto reductases of mammals and found that rat liver aldo-keto reductase is an aldehyde reductase which reduces as a sole exception the ketone substrate, daunorubicin. Studies on inhibitors and activators of the enzyme show that the use of inhibitors may have clinical application for the control of this enzyme system.

In studies of the pharmacodynamics of cancer chemotherapeutic agents, we have purified the microsomal reductive glycosidase and shown that this enzyme and NADPH cytochrome P450 are the same. Our studies of the ferric ion complex of adriamycin show that this drug complex may have clinical utility since in animal studies less drug is taken up by the myocardium. However, other studies do not indicate any difference in toxicity. Therefore additional studies may be of value in studying the ferric ion complex.

We have initiated a study of the mechanism of action of bleomycin. We have shown that bleomycin, a polypeptide, acts catalytically to oxidize ferrous ion to ferric ion. Our studies show that the bleomycin process conforms to Michaelis-Menton kinetics and leads to a possible mechanism of action, suggesting that bleomycin binds to DNA and catalyzes metal ion oxidation and oxygen reduction in the proximity of DNA, leading to DNA cell damage and cell toxicity. by a free radical reaction.

In studies on the cellular control mechanisms, we expanded our tissue culture and cell culture facility and are interested in the isolation of substances which affect the growth and differentiation of normal and malignant human cells. Our studies on the phytohemagglutinin isolectins have shown that L4 and E4 isolectins interact with plasma proteins leading to artifactual estimates of their interaction with white or red blood cells. In this study, we determined that phytohemagglutinins bind to human red cells and that both subunits have approximately the same number of binding sites, but with differing affinities. These isolectins have proven to be valuable as tools in the study of the normal physiology of human white cells and red cells.

Other studies in the area of cell biology and cellular control mechanisms were the use of laser cytometry in quantitation of lymphocyte transformation, passive immunization against B16 melanoma, and the cell mediated immune response in AKR lymphoma.

Among the clinical pharmacology activities of the Laboratory of Clinical Biochemistry was an attempt to develop a new rapid model for anthracycline induced cardiotoxicity. In this study we used the rapidly growing larva stage of an insect, *manduca sexta* or the tobacco horn worm. We showed that adriamycin in young *manduca sexta larva* leads to lesions in the dorsal vessel which are consistant with the type of damage seen in mammalian cardiotoxicity. The *manduca sexta* may ultimately prove to be a useful tool to investigate the toxicity of chemotherapeutic agents.

In our study of aspirin effects on lymphocyte blastogenesis, we have shown conclusively that aspirin has little effect on the response of lymphocytes to phytohemagglutinin, except at dosage levels approaching 40 mg/100 ml.

Finally, we have also developed a high dose, methotrexate-folic acid rescue pharmacokinetics model. This model has been useful to simulate the reaction of patients with impaired renal function.

Other projects have been terminated as their importance and utility have decreased.

Two new expanding technical functions in the Laboratory of Clinical Biochemistry have been the installation and development of analytical units in gas liquid-mass spectrometry and electron paramagnetic resonance spectrometry. These units are interfacing and supporting research in biochemical pharmacology, pharmacodynamics and clinical pharmacology.

In anticipation of moving to new quarters in midsummer, we have been restructuring our laboratory to a degree, and designing units which will be able to work together efficiently, cooperatively, among the BCRC group as well as between the University of Maryland and BCRC and DCT, NCI.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06012-08 LCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Biochemical Pharmacology of Antineoplastic and Other Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Chief	LCB, NCI
OTHER:	R. L. Felsted	Research Chemist	LCB, NCI
	H. Nakazawa	Visiting Fellow	LCB, NCI
	S. Pan	IPA	LCB, NCI
	M. Gee	Chemist	LCB, NCI
	R. Friedman	Chemist	LCB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.1	2.2	2.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Of mammalian cytoplasmic aldo-keto reductases, the rat liver aldehyde reductase is a soluble constitutive enzyme with a wide substrate spectrum. The only significant ketone substrate for this enzyme is daunorubicin. The amino sugar group is important in the mechanism of this reaction. "Site specific free radicals" are postulated as the active form of several anticancer antibiotics. The quinone antibiotics, adriamycin, daunorubicin, streptonigrin, and mitomycin C and the quinoid, actinomycin D are all activated to free radical state by mammalian microsomes or the microsomal enzyme, NADPH Cytochrome P 450 reductase. The free radical forms of these agents offer a new mechanism by which the DNA damage, other macromolecular damage, and cytotoxicity can be explained.

Project Description:

The anthracycline antibiotics, adriamycin and daunorubicin, the heterocyclic quinone antibiotics, streptonigrin and mitomycin C, and the benzoquinone, lapachol, are highly active quinone anticancer agents. We established that these quinone agents can be catalytically activated to a free radical state by a microsomal system requiring NADPH. The quinone containing anticancer agents augment the flow of electrons from reduced pyridine nucleotide to molecular oxygen. This enzymatic activity is inducible by phenobarbital and is found in microsomes of rat and mouse liver, heart, lung, spleen, and mouse L1210 and P388 tumors. NADPH-cytochrome C (P450) reductase isolated from rat liver microsomes retains the activities for both quinone augmented electron transport to oxygen and for free radical production with the quinone drugs. With NADPH-cytochrome C (P450) reductase, the Km values for oxygen consumption range from 0.4×10^{-4} M for 7-0-methyl nogalarol to 42.1×10^{-4} M for adriamycin $(Fe^{+++})^{4}$ complex. The pH optimum in phosphate buffer is 8.0. EPR spectrometry shows characteristic spectra for free radical forms of the quinone drugs during either the microsomal or the NADPH-cytochrome C (P450) reductase catalyzed reaction. We propose that the quinone drugs are activated to the free radical state intracellularly whereupon these "site-specific free radicals" associate selectively and preferentially with macromolecular intracellular structures causing damage and subsequent cytotoxicity.

We investigated the possibility that actinomycin D may be metabolized to a free radical with secondary formation of superoxide, by utilizing polarographic, spectral, chemical, and electron paramagnetic approaches to this problem. Rat liver microsomes in 0.2 M pH 8.0 phosphate buffer catalyze an NADPH dependent oxygen consumption which is dramatically stimulated by actinomycin D. The augmentation of oxygen consumption by actinomycin D is absolutely dependent on NADPH as cofactor and microsomal protein. Boiled microsomes are inactive. NADPH cytochrome P-450 reductase isolated from the liver microsomes also catalyzed the actinomycin D facilitated oxygen consumption. Kinetic studies of this reaction with both microsomes and NADPH cytochrome P-450 reductase follow classical Michaelis-Menten kinetics. Data analysis by Lineweaver-Burk plot gives an apparent Km for actinomycin D of 3.6×10^{-4} M. The actinomycin D enzyme intreaction stimulates the production of superoxide as shown by the stimulation of epinephrine oxidation. Superoxide dismutase inhibits the oxygen consumption. Electron paramagnetic resonance studies suggest that an actinomycin D free radical intermediate is produced in the reaction. Microsomes from other normal tissues and mouse tumors also catalyze the reaction. We propose that the activated free radical actinomycin D may be a "site specific free radical" which may be the pharmacologically active form of this anticancer agent in causing DNA damage and cell death.

Rat liver aldehyde reductase is a soluble constitutive enzyme having the ability to catalyze the reduction of several natural aldehydes such as lactaldehyde, glyceraldehyde, glyceraldehyde-3-phosphate, glucuronate, glucuronolactone, and succinic semialdehyde. However, on the basis of apparent Km, Vmax and relative pseudo-second order rate constants (k cat/Km), 4-carboxybenzaldehyde is the best substrate. The only significant ketone substrate is the antibiotic, daunorubicin, which is reduced with the unique alkaline optimum of pH 8.5. All other substrates (except adriamycin) were reduced at an acid optimum of pH 6.0 The N-acetylation of the free amino group of daunorubicin eliminates the alkaline activity suggesting the involvement of this group in the unique alkaline optimum. Reductase activity is noncompetitively inhibited by barbital and phenobarbital. Inhibitors of the classical alcohol dehydrogenase do not inhibit the aldehyde reductase. These properties suggest that this enzyme belongs to the class of reductases ubiquitously distributed among animal tissues and classified as alcohol:NADP$^+$ oxidoreductases (E.C. 1.1.1.2).

The elucidation of the biotransformations of antineoplastic agents are central to understanding the pharmacodynamics and toxicity of these agents. Additionally, understanding the metabolic machinery can be useful in manipulating the affects of the drugs.

Publications:

Felsted, R.L., Richter, D.R., and Bachur, N.R.: Rat liver aldehyde reductase. Biochemical Pharmacol. 26: 1114-1124, 1977.

Bachur, N.R., Gordon, S.L., and Gee, M.V.: Anthracycline antibiotic augmentation of microsomal electron transport and free radical formation. Molec. Pharmacol. 13: 901-910, 1977.

Aszalos, A.A., Bachur, N.R., Hamilton, B.K., Langlykke, A.F., Roller, P.P., Sheikh, M.Y., Sutpin, M.S., Thomas, M.C., Wareheim, D.A., and Wright, L.H.: Microbial reduction of the side chain carbonyl of daunorubicin and N-acetyldaunorubicin. J. Antibiotics 30: 50-58, 1977.

Loveless, H., Arena, E., Felsted, R.L., and Bachur, N.R.: Comparative mammalian metabolism of adriamycin and daunorubicin. Cancer Res. 38: 593-598, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06014-08 LCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cellular Control Mechanisms Affecting Cell Growth and Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Chief	LCB, NCI
OTHER:	M. J. Egorin	Clinical Associate	LCB, NCI
	R. L. Felsted	Research Chemist	LCB, NCI
	F. T. Sanel	Research Biologist	LCB, NCI
	R. N. Odders	Clinical Associate	LCB, NCI
	D. Ross	Clinical Associate	LCB, NCI
	A. Pollak	IPA	LCB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The isolation of substances from plant and animal sources which effect the growth and differentiation of normal and malignant human cells is paramount in the study of the processes which control cellular activities. By studying these materials as biochemical tools, we may be able to understand the biochemical mechanisms controlling cell proliferation and differentiation. Also, by investigating points where control of metabolic pathways are possible, we may find agents to control cellular growth, differentiation, and proliferation. Rat liver regenerative stimulator stubstance and normal hepatic regeneration are affected by a diurnal rhythm. Reversal of controlled lighting reverses these rhythms in normal animals. The phytohemagglutinin isolectins show different interactions with serum components leading to artifactual estimates of their interactions with white or red blood cells. Binding of isolectins to normal erythrocytes shows a constant number of binding sites but alteration of binding constant in the E subunits vary.

-6040
(Rev. 10-76)

Project Description:

The effect of a controlled lighting schedule on the activity of a weanling rat liver extract that stimulates DNA synthesis in regenerating adult rat liver, and on the response of the test animals to the extract was investigated. Both activity of the extract and endogenous DNA synthesis in the weanling animals follow the same distinct diurnal rhythm. Reversal of the lighting schedule reverses the rhythm of endogenous DNA synthesis but activity of the extract no longer correlates with the peak of DNA synthesis. Diurnal rhythm also has a striking effect on DNA synthesis in the regenerating test animal, but the extract increases DNA synthesis to the same relative degree, regardless of the time of day the hepatectomy is performed.

The ability of the red kidney bean (Phaseolus vulgaris) phytohemagglutinin isolectins L4 and E4 to transform human lymphocytes cultured in serum free or serum supplemented medium was studied. Previous studies done in fetal bovine serum supplemented medium have shown L4 to be 30-60 times more potent a mitogen than E4. In serum free conditions, this difference was much less, L4 being only 3-9 times more potent that E4. In serum free medium, optimal mitogenic concentrations of L4 and E4 were 1.1-3.3 ug/culture and 3.3-10 ug/culture respectively as compared to 3.3-10 ug/culture and 90-270 ug/culture for L4 and E4, respectively, in fetal bovine serum supplemented medium. L4 stimulated lymphocytes in serum containing medium transform more rapidly than do L4 treated cells cultured in serum free conditions. Fetuin added to serum free cultures of lymphocytes more effectively inhibited transformation induced by E4 than by L4. Although the binding of E4 and L4 to lymphocytes was greatly reduced by the addition of fetal bovine serum to the medium, the reduction in E4 binding with much greater that that in L4 binding. Neither L4 or E4 caused the death of lymphocytes cultured in serum free or fetal bovine serum supplemented medium. The results of earlier studies done in fetal bovine serum supplemented medium include serveral artifacts related to the differential interaction of phytohemagglutinin isolectins with fetuin and other serum glycoproteins.

The *Phaseolus vulgaris* isolectins L4, L3E1, L2E2, L1E3, and E4 were radiolabelled by the chloramine T method with Na^{125}IO$_4$, and their binding to human erythrocytes studied. A normal erythrocyte has approximately 8 x 10^5 receptor sites for each isolectin, however, the association constants (Ka) of binding increased from 1.1 x 10^7 M^{-1} to 3.8 x 10^8 M^{-1}, with increasing number of E subunits per tetrameric isolectin molecule. Isolectin to erythrocyte binding reached equilibrium rapidly and was reversed by fetuin. All isolectins competed with ^{125}I-E$_4$ for erythrocyte binding sites, with a constant, K$_I$, similar to the Ka calculated for each respective radiolabelled isolectin. When isolectin binding at 0^o, 4^o, or 8^o was compared to that at 25^o, there was no reduction in the number of binding sites per cell, but the Ka of E$_4$ was reduced to 3 x 10^7 M^{-1}. Fixed erythrocytes displayed similar isolectin binding characteristics.

We will continue our studies on the control mechanisms which affect cellular growth and differentiation, and attempt to elucidate control principles affecting all levels of cellular machinery. We hope to elucidate the mechanism of action of the isomitogens on cell membranes. We will continue our search for materials characteristic of altered metabolic pathways, which maybe used to quantify malignant cells *in vivo*.

Publications:

LaBrecque, D.R., Feigenbaum, A., and Bachur, N.R.: Diurnal rhythm: Effects on hepatic regeneration and hepatic regenerative stimulator substance. *Science* 199: 1082-1084, 1978.

Egorin, M.J., Litvin, F., Felsted, R.L. and Bachur, N.R.: Phytohemagglutinin isolectin stimulation of human lymphocytes cultured in serum free medium. Life Science, June, 1978.

Egorin, M.J., Odders, R.N., Sanel, F.T., Felsted, R.L., and Bachur, N.R.: Inhibitory effects on phytohemagglutinin isolectins L4 and E4 on L1210 cells. Cancer Res. 38: 1677, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06016-08 LCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Pharmacodynamics of Cancer Chemotherapeutic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Chief	LCB, NCI
OTHER:	M. J. Egorin	Clinical Associate	LCB, NCI
	R. N. Odders	Clinical Associate	LCB, NCI
	W. Caspary	IPA	LCB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on the mammalian microsomal reductive glycosidase show that this enzyme and NADPH cytochrome P450 reductase are the same. The ferric ion complex of adriamycin shows similar action to adriamycin in L1210 cell uptake and inhibition of DNA synthesis. However, the metabolism of the compound is decreased, and the distribution in BALB/c mice shows less uptake by muscle, kidney, and heart. Bleomycin A2 binds to and breaks DNA. We find bleomycin catalyzes the oxidation of Fe^{++} to Fe^{+++}. We therefore suggest that bleomycin A2 is an enzyme (ferrous oxidase) and may catalyze metal ion oxidation and oxygen reduction in the proximity of DNA leading to DNA damage and cell toxicity.

PHS-6040
(Rev. 10-76)

Project Description:

Microsomes contain an enzyme which reductively cleaves the glycoside bond of the anthracycline antibiotics adriamycin and daunorubicin. We solubilized and purified this reductive glycosidase from rat liver microsomes by means of enzymatic digestion and successive fractionation on Sephadex G100 and DEAE cellulose columns. The procedure yields a 600 fold purification, an enzyme which is homogeneous by disc gel electrophoresis, and a 30% recovery of the starting enzymatic activity. Gel filtration of the enzyme on calibrated Sephadex columns gives a molecular weight approximating 74,500 Daltons. The reductive glycosidase has a broad pH optimum from 7.0 to 8.5 and depdends on NADPH, whereas other pyridine cofactors do not support the reaction. Each mole of reductive glycosidase contains one mole each of FMN and FAD. Oxygen specifically and reversibly inhibits the reaction. Over 90% inhibition is obtained when reaction solutions are equilibrated with normal air. Nitrogen or argon reverse the oxygen induced inhibition. Other anthracycline glycosides which are reductively cleaved by the enzyme are adriamycinol, daunorubicinol, *N*-acetyldaunorubicinin, and carminomycin. In addition to being inhibited by Fe^{++}, Cu^{++}, and Zn^{++}, the enzyme is inhibited by parachloromercuribenzoate (1 x 10^{-5} M). The enzyme is identical to NADPH cytochrome P450 reductase.

A ferric ion complex of adriamycin (FIC) has been reported to have decreased animal toxicity at doses equally efficacious with adriamycin. L1210 cells incubated with adriamycin or FIC (1 to 5 uM) for 2 hours at 37^{o} are associated with a similar amount of drug fluorescence even though there is greater initial binding of FIC to cells. At higher concentrations (8-10 uM), less fluorescence is associated with cells incubated with FIC. DNA synthesis as measured by ^{3}H thymidine incorporation is inhibited to a lesser degree by the FIC than by adriamycin at concentrations of 2-10 uM. *In vitro* metabolism of FIC by aldo-keto reductase or reductive glycosidase is *markedly* decreased compared to adriamycin metabolism. Distribution of adriamycin and FIC in BALB/c mice 1 hour after an IV injection of 16 mg/kg of drug was studied by measuring total fluorescence due to drug and metabolites in various tissues. Fluorescence associated with the lung was significantly higher (p greater than 0.01) while that associated with the heart, kidney, and muscle was significantly lower (P less than 0.01) after FIC administration. These findings suggest that the increased therapeutic index of the FIC over that of ADR is due, at least in part, to changes in uptake, distribution, and metabolism brought about by the noncovalent binding of ferric ion to adriamycin.

The bleomycins are polypeptides produced by *Streptomyces verticullus* and are used in chemotherapy of various malignancies. The bleomycins bind to DNA and cause DNA strand breaks. Bleomycin A$_2$ is the major component of the multicomponent drug used clinically. We find that bleomycin A$_2$ catalytically oxidizes Fe^{++} to Fe^{+++} as monitored by an oxygen electrode. Oxygen is reduced during this process and classical Michaelis-Menton kinetics are followed (Vmax = 0.27 mM/sec, km = 1.8 mM). One mole of bleomycin A$_2$ turns over approximately 5000 moles Fe^{++}/min. The catalytic activity of bleomycin A$_2$ stimulation has a pH optimum between pH 6 and 7 in 0.1 M maleate. The enzyme substrate complex can be observed spectrophotometrically. The visible u.v. spectrum of the product is identical to that produced by the addition of Fe^{+++} to bleomycin A$_2$. Based on these data, we suggest that bleomycin A$_2$ is an enzyme and can be classified as a ferrous oxidase. Since bleomycin binds to DNA, the antibiotic may catalyze metal ion oxidation and oxygen reduction in the proximity of DNA leading to DNA damage and cell toxicity.

To investigate the biochemical and physiological effects of antineoplastic drugs and their mechanism of action. To correlate the effects and activities of the drugs with their chemical structures.

Understanding the structure activity relationships of drugs is useful in planning new improved agents. Further explanations through study of the pharmacodynamics can help clarify problems in chemotherapy.

Publications:
None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06241-08 LCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of Antineoplastic and Other Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Chief	LCB, NCI
OTHER:	M. J. Egorin	Clinical Associate	LCB, NCI
	F. T. Sanel	Research Biologist	LCB, NCI
	R. H. Goebel	Research Associate	LTB, NCI
	M. Berman	Chief	LTB, NCI

COOPERATING UNITS (if any)

Clinical Oncology Branch, Baltimore Cancer Research Center, NCI
Laboratory of Theoretical Biology, NCI, NIH

LAB/BRANCH
Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our sustained investigation of the clinical pharmacology of antineoplastic agents continues to aid in the understanding of clinical problems through fundamental approaches. In an attempt to develop a new efficient rapid model for anthracylcine - induced cardiotoxicity we studied Manduca Sexta (tobacco horn worm) for effects of anthracycilnes on larvae growth, development, and heart histopathology. Invertebrate models may be useful in analog testing. Our studies of aspirin effect on lympohcyte transfromation show that the widely used drug has little effect on lymphocytes from normal donors except at very high doses (40 mg%). A component of RPMI 1640 media inhibited this effect. High dose methotrexate folinic acid rescue pharmacokinetics were studied and a pharmacokinetic model was developed. The model can be used to simulate the kinetics of methotrexate in patients with impaired renal function.

Project Description:
Although aspirin inhibits the response of cultured lymphocytes to phytohemagglutinin, it is controversial whether lymphocytes from aspirin-treated humans respond normally to phytohemagglutinin. We treated 12 volunteers with aspirin at levels of 600 or 900 mg four times a day. Their lymphocytes were isolated before and during therapy and one week after the cessation of therapy, cultured in RPMI 1640 medium or minimal essential medium (MEM) with purified phytohemagglutinin isolectins, and assessed for ^{3}H thymidine incorporation and glucose utilization. Lymphocytes from normal donors were also purified and cultured with aspirin in RPMI 1640 medium, MEM, or autologous plasma and assessed under phytohemagglutinin isolectin stimulation. The lymphocytes from aspirin-treated volunteers responded normally to L4 and E4 phytohemagglutinin whether obtained during aspirin treatment or not and regardless of the culture medium used. However, addition of up to 40 mg/100 ml of aspirin to L4-stimulated cultures produced a dose-dependent inhibition of blastogenesis and glucose utilization. This inhibition was substantially more pronounced in MEM than in RPMI 1640 or plasma. Supplementation of MEM with amino acid and vitamin components of RPMI 1640 failed to reduce the degree of aspirin inhibition. Aging of RPMI 1640 reduced its protective effect agains aspirin inhibition, indicating that the responsible factor was labile. Salicylate did not affect the binding of L4 to lymphocytes in either medium.

The infusion of high doses of methotrexate followed by folinic acid rescue is clinically useful against a variety of tumors. We studied the plasma pharmacokinetics of high-dose methotrexate infusions in patients with advanced cancer and devised a compartmental, kinetic model. Our model is based on an earlier, mathematical model which describes the pharmacokinetics of moderate-to-high-dose methotrexate given as a single, intravenous injection. Mathematical equations for our model were solved on a UNIVAC 1108 computer with the SAAM program. Seven compartments represent the distribution spaces for methotrexate and its metabolites. The transport of drug into and out of compartments is described by first-order differential equations. A nonlinear, concentration-dependent function is used for renal excretion with saturation of secretory and reabsorption mechanisms by methotrexate. Our model accurately depicts the pharmacokinetics of nine courses of therapy in five patients. The model can also be used to simulate the kinetics of methotrexate for patients with impaired renal function.

Adriamycin is a chemotherapeutic agent with demonstrated anti-cancer properties but with serious side effects of cardiotoxicity in man and most vertebrates studied. Invertebrate models have not been investigated to test such agents. It appeared logical to look at the dorsal vessel (a single-layered striated muscle heart analog in insect larva) in order to provide a short term model of adriamycin induced cardiomyopathy. Adriamycin specific fluorescence facilitated localization of the drug. Fifth instar larvae were injected with single and multiple doses of 0.5 and 1.0 mg of adriamycin and tissues were processed for fluorescence and electron microscopy. At these doses, adriamycin profoundly affected larval development; few pupated and metamorphosed to adult moths. Five to 15 minutes after adriamycin injection, dorsal vessels, pericardial cells, gut, and Malpighian tubules exhibited storng fluorescence. Earliest lesions in the dorsal vessel noted within one hour, consisted of focal misalignment of myofilaments, vacuolization, and increased lucency of the cytoplasmic matrix. After four days cytotoxicity was marked. Z lines were disrupted, myofilaments were disarrayed and in some instances displaced by dense pleomorphic inclusions and large lipid droplets. Normally thick basement membranes bordering the sarcolemma had thinned and detached except where held by hemi-desmosomes. Mitochondria, normally concentrated in the lateral out-pocketings of the sarcolemma were scattered and appeared to be diminished and condensed. SER cisternae were distended and membranes were fragmented. Many of these lesions are similar to those observed in human cariomyopathy after prolonged treatment with adriamycin. These studies suggest taht Manduca Sexta is a useful tool to investigate the toxicity of an important chemotherapeutic agent, and that invertebrate models may be valuable substrates for the evaluation of pharmaceuticals.

Publications:

Egorin, M.J., Felsted, R.L., and Bachur, N.R.: Salicylate effects on the response of human lymphocytes to phytohemagglutinin isolectins. Clin. Immunology and Immunopath. 10: 1-10, 1978

Reich, S.D., Bachur, N.R., Goebel, R.H., and Berman, M.: A pharmacokinetic model for high dose methotrexate infusions in man. J. Pharmacokin. and Biopharmaceutics 5: 421-433, 1977.

Riggs, C.E., Benjamin, R.S., Serpick, A.A., and Bachur, N.R.: Biliary disposition of adriamycin. Clin. Pharmacol. Therap. 22: 234-241, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06969-01 LCB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Laser Cytometry in Quantitation of Lymphocyte Transformation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Shu	Staff Fellow	LCB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro lymphocyte activation by mitogens and antigen was analyzed by laser transmission cytometry. The new method directly measures cellular events. The formation of lymphoblasts and increase in total cells are quantitatively related to conventional methos of pulse labelling. Statistically this approach appears to be more reproducible. Immunotherapeutic effect against B16 melanoma was evaluated. Heterologous antibodies against B16 cells produced in rabbits had an indirect immunofluorescence titer of 1280. Passive immunization of C57BL/6 mice reduced tumor incidence from 100% in the controls to 21.2% (7/33). The mechanism of the therapeutic effect is not related to complement mediated cytotoxicity, since the antibodies failed to fix mouse complement in vitro.

PHS-6040
(Rev. 10-76)

Project Description:

In vitro lymphocyte transformation by mitogens (CPHA, ConA) and an antigen (SKSD) was analyzed by laser transmission cytometry. The kinetics of the formation of lymphoblasts over a period of 8 days closely paralleled the amount of thymidine incorporation. However the steady increase of total cells in stimulated cultures concomitant with the decrease of ^{3}H-thymidine uptake indicated discordance between the actual cell proliferations and the utilization of exogenous thymidine. Analyses of mitogen and antigen dose responses by current criteria revealed that there were at least two mechanisms involved in high dose suppression observed by pulse labeling, a specific suppression of blastogenesis and a nonspecific cytotoxic effect. In addition to direct examination of cellular event during blastogenesis, the method appeared to be more reproducible than ^{3}H thymidine incorporation assay.

Immunotherapeutic effect against B16 melanoma was observed when heteroantibodies were administered intraperitoneally at days -1, 0, 1 in relation to subcutaneously tumor transplant of 1 x 10^5 cultured B16 cells/mouse. The antibodies against B16 melanoma cells produced in rabbits had an indirect immunofluorescence titer of 1280. Futher characterization of the antibodies revealed that they also exhibited cytotoxic effects on target cells *in vitro* with rabbit or guinea pig complement but not with C57BL/6 mouse complement. Passive immunization of the susceptible hosts reduced tumor incidence from 100% in the control to 21.2% (7/33). The protective effect of the heteroantibodies was significantly better than the active immunization with irradiated tumor cells. The active immunization afforded a reduced tumor incidence down to 68% (15/22) at 4 weeks after tumor challenge. The therapeutic effect was not due to complement dependent antibody cytotoxicity since the Winn test did not show significant reduction in tumor incidence. Experimentas are currently carried out to characterize the specificity, dose response, time course, and the effect on tumor metastasis by the passive immunization.

Publications:

Halubar, K., Albini, B., Shu, S., and Wolff, K.: Enzyme antibody methods in immunodermatopathology in Immunopathology of the skin: Labeled antibody studies. (Beutner, E.H., Chorzelski, T.P., Bean, S., and Jordon, R.E., eds). Dowden, Huchinson, and Ross, Straudsbourg, PA, Chapter 4, 1978.

Shu, S., Cimino, E.F., and Mardiney, M.R.: Light transmission analysis of lymphocyte activation by mitogens: A new technique. J. Immunol. Meth., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06992 -01 LCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cell Mediated Immune Response in AKR Lymphoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Hsu	Staff Fellow	LCB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Normal resident peritoneal macrophages from preleukemic AKR mice show considerable cytotoxicity against syngeneic AKR lymphoma cells but show little activity against L1210 and P388 cells. The cytotoxicity is inhibited in the presence of autologous serum. Macrophages obtained from C57B1/6 and Balb-C mice show no cytotoxicity against AKR lymphoma cells. The macrophage cytotoxicity is augmented by rabbit anti-mouse thymocyte serum, thus account for the increased survival of AKR mice that received the antibody. The macrophage cytotoxicity declines as the mice aged to 6 months or older. Mice bearing large transplanted subcutaneous lymphoma have macrophages which are totally inactive against syngeneic lymphoma.

PHS-6040
(Rev. 10-76)

Project Description:
The transplantable AKR lymphoma has been maintained by weekly transplantation by subcutaneous or intraperitoneal injection to 2 month old AKR mice. All mice so transplanted with 10[illegible] cells die of widespread lymphoma during the 2nd and 3rd week. Active immunization with irradiated, neuraminidase treated or ConA coated lymphoma cells proved to be unsuccessful. However, mice which received injection of rabbit antimouse thymocyte globulin (RAMTG) show approximately 100% increase in mean survival time although no cure was observed.

The normal resident peritoneal macrophage from 2 month old AKR mice showed considerable cytotoxicity against the syngeneic lymphoma cells. The macrophage mediated cytotoxicity declined with the age of mice and the macrophage obtained from mice bearing large transplanted lymphoma showed little activity. Cell contact is a strict requirement for the lysis of lymphoma cells, as studied by the release of ^{3}H thymidine from the lymphoma cells. The cytotoxicity of macrophage is abrogated by the autologous serum at 1/2 dilution. The macrophages obtained from C57B1/6 and Balb-C mice show little or no activity against AKR lymphoma cells. The AKR macrophage has little or no cytotoxicity against other lymphoma cells such as L1210 and P388 cells. The reason for this specificity is unclear and whether there is a histocompatibility restriction or there is a distinction between the cell surface of lymphoma induced by virus and carcinogens remains to be investigated.

The macrophage mediated cytotoxicity against AKR lymphoma cells is greatly stimulated by rabbit antimouse thymocyte globulin (RAMTG). The RAMTG by itself stimulates the proliferation and thymidine uptake of lymphoma cells. Yet when RAMTG was injected together or separately with lymphoma cells into the 2 month old AKR mice, the growth of lymphoma was significantly retarded and the mean survival time was increased by about 100%. Thus the effect of RAMTG in vivo may be due to macrophage mediated cytotoxicity. The lymphoma cells could also deactivate macrophage cytotoxicity if present in excess. The present research stresses the important role of macrophage in immune defense against cancer cells. Further investigations will be directed to characterize the serum inhibitors, mechanism of macrophage specificity, and the reciprocal interaction between macrophage and the lymphoma cells. The observations with RAMTG also raises the possibility of using antilymphocyte serum in treating human lymphoma or lymphatic leukemia.

SUMMARY REPORT

LABORATORY OF MOLECULAR BIOLOGY
October 1, 1977 through September 30, 1978

One major area of concentration within the Laboratory of Molecular Biology has been the study of cellular regulation particularly as it effects those factors which control the stabilization of messenger RNA (mRNA) in normal and neoplastic tissues. In this connection, the significance of polyadenylic acid (poly(A)), a structure found at the 3' terminus of many mRNAs as well as viral RNAs, has been the subject of an intensive study in this Laboratory. Previously, we had shown that polyadenylic acid is a competitive inhibitor of many ribonucleases and more recently, an attempt has been made to study the function of poly(A) at the termini of mRNAs within three specific tissues: bovine muscle, amphibian and avian livers. These tissues were chosen because preliminary studies have indicated that they contain a wide assortment of RNases, all of which are capable of degrading mRNA. In each case, the enzymes in the tissues were isolated and characterized, and a particular mRNA from each of the tissues has also been isolated. The reasoning being that if the mRNA were stable in the presence of enzymes to which they would theoretically be exposed, then a reasonable model could be constructed to examine the mechanisms of polyadenylic acid action. Thus, it was found that if polyadenylic acid were removed from mRNA and the messenger exposed to ribonucleases from the same tissue, the messenger would be rapidly degraded. On the other hand, if the mRNA containing a full complement of poly(A) were exposed to the same enzyme, no degradation took place. An important factor in the stabilization of mRNA then would appear to be the presence of polyadenylic acid at its 3' terminus.

In addition to what has been discussed above, the finding that poly(A) can effect a change in the secondary structure of the RNA to which it's linked, is also of considerable interest. The polynucleotide, in reducing secondary structure of a mRNA can aid in allowing the messenger to be "read" more readily as the messenger traverses the ribosome. In effect, this structure acts as a mechanism which enables mRNA to be stabilized but at the same time maintains linearity in the mRNA to allow for a more efficient translation of genetic expression.

The Laboratory, also concerned with other aspects of cell regulation, is continuing its study of the relationship between oncogenic viral proteins and cell transformation. In this respect, the relationship between the major internal protein of the virion core (p30) and reverse transcriptase is of particular importance. Our observations that p30 and reverse transcriptase can be isolated from Rauscher leukemia virus as a complex and that the complex is present in the thymus and spleen of AKR mice at the onset of leukemia, all suggest that the formation of this complex may be an important prerequisite in viral assembly. We are continuing these experiments and have isolated and purified other proteins within the virion core (that is p10, p12 and p15) to study more completely viral assembly and how that is related to oncogenesis.

Transformation is also being examined in Herpes simplex virus as well. In these studies, our purpose is to understand the mechanism by which transformation of human cells can occur. We have selected cervical cancer as a mechanism through which viral transformation can be studied. Herpes virus has been implicated in cervical cancer.

Our studies will attempt to examine if cell-free transcripts produced from Herpes can be found in cells transformed in cervical cancer.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CM 06242-08 LMB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Regulation of Poly(A) Induced RNase Inhibition by Metals or Polyamines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Karpetsky	Sr. Investigator	LMB, NCI
OTHER:	C.C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Polynucleotdie phosphorylase was used to covalently link poly(A) tracts having 5, 18, 31, 41, 59, 73, 100 and 150 residues to the 3' termini of *E. coli* 5S [^{3}H] rRNA (5S [^{3}H] rRNA $(A)_n$). Upon hydrolysis of these polyadenylated polynucleotides by ribonucleases from *Citrobacter* sp., *Enterobacter* sp., bovine pancreas, human spleen and human plasma, it was found that the $(A)_n$ segment inhibited RNase activity, and that the inhibition was enhanced as the polyadenylic acid tract was lengthened. Certain polyamines, such as spermidine restores enzyme activity in the presence of 5S rRNA $(A)_n$ (n=73). Low concentrations of antimalarial compounds containing polyamine substratuctres, such as Atabrine (a 9-aminoacridine) or Plaquenil (a 4-aminoquinoline), also reverse $(A)_n$ induced inhibition of RNase activity while 8-aminoquinolines such as Primaquine were ineffective. Divalent metal ions (mg^{2+}, Mu^{2+}, Ni^{2+}, or Co^{2+}) also overcome inhibition, however, the action of metals may be distinguished from that of polyamines because in the absence of inhibition, 5S rRNA·$(A)_n$ is hydrolyzed at a faster rate than 5S rRNA only in the presence of metal ions. In all systems studied, inhibition of RNase activity mediated by 5SrRNA-$(A)_n$ is regulated by the chain length of the poly(A) segment, the concentrations of enzyme and substrate and the identity and concentration of metal ions or polyamines.

PHS-6040
(Rev. 10-76)

The inhibition of ribonuclease (RNase) activity by either free polyadenylic acid ($(A)_n$) or $(A)_n$ covalently linked to the 3' termini of RNA substrate molecules is described in another report (Fnctional Significance of Polyadenylic Acid). It was of interest to determine if polyamines or certain polyamine analogs could reverse this inhibition. Utilization in this study were purified RNases from five diverse sources: Citrobacter sp., Enterobacter sp., bovine pancreas, human spleen and human plasma. $(A)_n$ was covalently linked to 5S $[^3H]$ rRNA using polynucleotide phosphorylase. 5S $[^3H]$ rRNA $(A)_n$ having an average of 5, 18, 31, 41, 59, 73, 100 or 150 adenylic acid residues at the 3' termini were prepared in this manner. Addition of low concentrations (3×10^{-5} M) of spermidine to reaction mixtures containing an RNase and 5S $[^3H]$ rRNA $(A)_n$ resulted in increases in enzyme activity that varied with the identity of the enzyme. Inhibition was totally reversed for RNase A or human plasma RNase while partial reversal was found for the enzymes from human spleen or bacteria. Although the rate of RNase mediated hydrolysis of non-polyadenylated substrates such as yeast RNA or poly(C), decreases upon addition of 5S rRNA $(A)_n$ to the reaction mixture, spermidine reverses this effect. Increases in the length of the 3'-$(A)_n$ segment necessitated the addition of larger quantities of spermidine to achieve the same enzyme activity. The structure of $(A)_n$ gives certain clues regarding the types of polyamines that may regulate inhibition of RNase activity. Incorporation of a polynuclear aromatic residue into the structure of spermidine, for example, should result in a compound that can both bind to the phosphate moieties present in RNA and $(A)_n$ as well as intercalate with the single stranded partially stacked structure that $(A)_n$ assumes at neutral pH. Accordingly, polyamines incorporating the following substructures were tested using an RNase and 5S $[^3H]$ rRNA$\cdot(A)_n$ having an average of 100 adenylic acid residues at the 3' termini: 9-aminoacridines; 4-ammoquinolines; 8-ammoquinolines. All compounds tested have been or are currently in use as antimalarial drugs. Our results indicated that the 8-aminoquinolines tested (Primaquine, Pentaquine, Isopentaquine, Quinocide) did not reverse inhibition (10^{-6} - 5×10^{-4} M). However, 5×10^{-5} M Amodiaquine and Plaquenil (4-aminoquinolines) completely restored enzyme activity. Lower concentrations (5×10^{-6} - 10^{-5} M) of Acranil, Azacrine or Atabrine were similarly efficatious. With the exception of the 9-aminoacridines, none of these compounds stimulated or inhibited the RNase mediated hydrolysis of 5S $[^3H]$ rRNA. Compounds such as Atabrine, however, at concentrations $> 5\times10^{-5}$ induce > 50% inhibition of substrate hydrolysis. The three classes of compounds are therefore, quite distinct in their action: 8-aminoquinolines neither reverse $(A)_n$ induced inhibition nor inhibit substrate hydrolysis; 4-aminoquinolines reverse inhibition but do not interfere with degradation of substrate; 9-aminoacridines, on the other hand, are superior to 4-aminoquinolines in terms of reversal of $(A)_n$ induced inhibition, but as the concentrations of the acridines are increased, substrate hydrolysis becomes increasingly difficult. Binding studies of Atabrine, Plaquenil or Primaquine to $(A)_n$, 5S rRNA or RNase A revealed that drugs such as Atabrine (having 3 aromatic rings and 2 positive charges/molecule) had a greater affinity for either $(A)_n$ or 5S $[^3H]$ rRNA than does primaquine (2 aromatic rings and 1 positive charge/molecule). Thus, binding to polynucleotides and efficacy in reversing $(A)_n$ induced inhibition of RNase activity appear to be linked. However, distinct types of complexes appear to be formed with the polynucleotides and either 4-aminoquinolines (Plaquenil) or 9-aminoacridines (Atabrine). A similar quantity of Atabrine is bound to either $(A)_n$ or 5S $[^3H]$ rRNA. Theresults for Plaquenil are similar although a lesser quantity of this drug is bound to either polynucleotide. However, Plaquenil does not inhibit RNase mediated hydrolysis of 5S $[^3H]$ rRNA while Atabrine does, suggesting that the complex between Atabrine and 5S $[^3H]$ rRNA is distinct from, and more resistent to RNase mediated hydrolysis than the Plaquenil-5S $[^3H]$ rRNA complex. Metal chlorides in low concentrations (2×10^{-5} M) $MgCl_2$, $MnCl_2$, $CoCl_2$ and $NiCl_2$ also reverse inhibition. $ZnCl_2$ was moderately efficatious while $CuCl_2$ inhibited the hydrolysis of substrate. There was one major difference between the polyamine or metal induced reversal of $(A)_n$ inhibition ofRNase activity. Use of polyamines resulted only in a restoration of enzyme activity while metals yielded a large enhancement of activity. Thus, comparing RNase reaction mixtures that contain either 5S $[^3H]$ rRNA and metal ion or 5S $[^3H]$ rRNA$\cdot(A)_n$ and metal ion, the activity for the latter case was 40% more than that of the former. This would appear to result from the structural destabilization produced in 5S $[^3H]$ rRNA concomitant with covalent addition of 3'-terminal $(A)_n$ segments. The above results indicate that the inhibition of RNase activity by 3' terminal tracts of$(A)_n$ is subject to regulation by low concentrations of certain polyamines and metals. It is interesting to speculate that one of the consequences of administration ofcertain antimalarials may be the regulation of RNA hydrolysis. In the future, studies will concentrate on the effect of the drugs and metals on RNA structure in further attempts to understand the interrelation of structure and activity in RNA-RNase interactions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06933-03 LMB

PERIOD COVERED
October 1, 1977 to September 31, 1978

TITLE OF PROJECT (80 characters or less)

Ribonuclease Activities in Human Sera

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Karpetsky	Sr. Investigator	LMB, NCI
OTHER:	C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Polyadenylic acid (poly(A)) was incorporated into polyacrylamide gels and utilized as a substrate for human serum ribonucleases after electrophoresis. This was accomplished by selecting conditions for electrophoresis such that the enzymes had no activity. After electrophoresis, gels were incubated in buffer to induce hydrolysis of poly(A) in the narrow regions to which the RNases migrated. Staining revealed these regions of enzyme activity. Eight bands of activity were consistently found in the sera of 19 volunteer blood donors. All samples from this group gave a band of activity at $R_f = 0.39$. This enzyme activity was totally absent in the sera of 13. Patients having kidney disease of varied etiology (C_{CR} 5 ml/min.). However, all of these patients had a distinct activity at $R_f = 0.33$, not found in sera from normal individuals. Although the activity patterns measured using sera of patients having multiple myeloma differ from the normal pattern, the activity associated with decreased renal function was found in 3/3 patients having renal insufficiency concomitant with myeloma. Other patients (8/9) with higher C_{CR} values, had only the activity in this region found in normal volunteers. Currently, an activity band useful in the detection or grading of various types of tumor is being sought.

It was found previously that an inverse correlation exists between the degree of renal impairment (expressed as creatinine clearance, C_{CR}) and human serum ribonuclease (RNase) levels. Concentrations of RNase do not serve as a biomarker for cancer although levels are elevated in cancer patients having decreased glomerular filtration rates. Immunologic testing demonstrated that one enzyme accounts for greater than 95% of the RNase activity present in human sera. This enzyme hydrolyzes poly(C) (100%) or poly(U) (1%) but has no measurable activity against polypurines (poly(A), poly(G)). Attempts to determine if RNases normally present in very low amounts in sera, could be used as indicators of the presence, extent, or response to therapy of certain types of cancer or kidney disease, led to the development of a sensitive technique using polyacrylamide gel electrophoresis for visualization of these enzyme activities. Using gels containing poly(A), serum samples were electrophoresed under conditions in which the enzymes were inactive. Gels were then treated to permit hydrolysis of poly(A) to occur in the very narrow regions throughout the gel where RNases were present. Staining resulted in colored gels having clear regions corresponding to RNase activities. Eight bands of activity were consistently found in the sera of 19 normal volunteer blood donors (R_f = 0.08, 0.12, 0.19, 0.24, 0.31, 0.39, 0.52, 0.70). Thirteen patients having C_{CR} $<$ 5 ml/min. and requiring hemodialysis twice, and more often, three times a week, all lacked the activity band at R_f = 0.39 (range: 0.37-0.44). However, these patients with severe renal failure of varied etiology all had a new RNase activity band (R_f = 0.33, range: 0.32-0.34) not present in the sera of normal individuals. A large ($>$ 100%) increase in the enzyme aactivity present at R_f = 0.08 was also found in the sera of kidney disease patients relative to normal controls. Further evidence for the appearance of an activity band near R_f = 0.33 with the concomitant disappearance of the R_f = 0.39 band was found upon examination of sera from pretreatment multiple myeloma patients, some of whom also had renal insufficiency. The three patients with the lowest creatinine clearance rates, for example, all had the 0.33 R_f band and lacked the activity at R_f = 0.39. The reverse situation regarding the bands between R_f = 0.33-0.39 was found for 8/9 multiple myeloma patients having higher C_{CR} values. Thus, a specific RNase activity band is associated with renal insufficiency, and is not found in sera from normal volunteers or multiple myeloma patients not suffering concomitant renal failure. The relationship between the activity band that disappears (R_f = 0.39) and the one that appears (R_f = 0.33) as the rate of glomerular filtration decreases is not known. The two enzyme activities could be independent or one may derive from the other by molecular modification. Studies are currently underway to clarify this point. Although the banding pattern in the region R_f = 0.05-0.30 is different for sear obtained from multiple myeloma patients than the pattern found from normal individuals, there is a wide variation in the amounts of enzyme activity in each band in the former population. Future studies will attempt to relate specific banding patterns to the grate or stage of disease. Sera from patients with leukemia (acute and chronic myelogenous or lymphocytic) with or without renal insufficiency are also under examination. Sera from individual patients obtained during diagnosis, treatment, and remission will be used to obtain activity patterns. These experiments should clarify the utility of "activity" gels in determining the presence, extent, or response to therapy of specific tumors.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06965-02 LMB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Analysis of DNA Damage in vivo by Alkylating Agents and Ionizing Radiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Hilton	Visiting Associate	LMB, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A relation between the extent of DNA crosslinking and its alkaline elution properties has been established in L1210 cells in culture exposed to nitrogen mustard. This reaction allows a quantitative study of in vivo L1210 DNA crosslinking in tumor bearing mice exposed to the synergistic antitumor combination of Adriamycin and cyclophosphamide. The results indicate that a reduced ability to remove interstrand DNA crosslinks in cells exposed to the combination is responsible for the synergistic response.

[illegible]-6040
(Rev. 10-76)

The Adriamycin-Cyclophosphamide combination which shows potentiation in a variety of animal models and is employed clinically has been studied in the mouse L1210 leukemia system. Information available from earlier studies of our own and from the literature indicated that in the Adriamycin-Cyclophosphamide combination we were dealing with compounds which caused DNA strand breaks and DNA crosslinks, respectively. This simple picture quickly became complicated by the observation of extensive protein-DNA and DNA-DNA crosslinks in the DNA of tumor cells from Adriamycin treated mice. We have established that when given alone, at drug exposures which give little increase in life span in tumor bearing mice, the early strand breaks and DNA crosslinks induced early after Cyclophosphamide or Adriamycin treatment are cured extensively within 24 hours. In combination, however, there occurs a marked persistence of DNA cross-links which exceed the sum of the individually induced levels by two or three fold. The largest potentiation is found in the extent of DNA-DNA crosslinks.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CM 06977-01 LMB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

NMR Study of the Interactions of RNases and Polynucleotides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Ho	Sr. Investigator	LMB, NCI
OTHER:	T. Karpetsky	Sr. Investigator	LMB, NCI
	C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project was aimed at the synthesis of nucleotide analogs; the studies of the interaction between nucleotide analogs and RNase; to observe the chemical shift in the NMR spectrum of the RNase linstidine protons which are involved in the active site of the enzyme.

PHS-6040
(Rev. 10-76)

Synthesis of 6-d-ADP was achieved. Polymerization of 6-d-ADP by polynucleotide phosphorylase should afford the analog of poly(A). NMR study of the interaction of the analog of poly(A) and RNase should be able to reveal the characteristics of the binding. It should be easy to tell whether the active site of RNase enzymes are involved in the binding, or other binding sites of the RNase were actually involved.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06978-01 LMB

PERIOD COVERED
October 1, 1977 through Separtment 30, 1978

TITLE OF PROJECT (80 characters or less)

Stability of 5S RNA with Poly(A) Taleand 5S RNA with Poly(A) in Xenopus Laevis Oocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Ho	Sr. Investigator	LMB, NCI
OTHER:	G. Davies	Clinical Associate	LMB, NCI
	T. Karpetsky	Sr. Investigator	LMB, NCI
	C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aims of the project are to find whether poly(A) stabilizes 5S RNA in Xenopus laevis oocytes.

PHS-6040
(Rev. 10-76)

Project Description:

$[^3H]$ labelled 5S RNA was prepared by incubation of *E. coli* K_{12} with uridine $[5,6\text{-}^3H]$. 5S RNA ($[^3H]$) was injected, together with different proportions of poly(A), length 54, into oocytes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06979-01 LMB

PERIOD COVERED

October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Isolation and Purification of mRNA from Bovine Pancreas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Ho	Sr. Investigator	LMB, NCI
OTHER:	G. Davies	Clinical Associate	LMB, NCI
	C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary aims of this project are the isolation and purification of messenger RNA from bovine pancreas, the stability of the messenger RNA under a variety of conditions against RNase, and the comparison of the behavior of messenger RNA with or without poly(A).

Attempts were made to isolate and purify the messenger RNA from bovine pancreas by phenol-chloroform extraction and purification of messenger RNA from the rest of RNA by oligo-DT column chromatography. All attempts failed to afford the desired mRNA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06980-01 LMB

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)
Study of the Physiological Importance of Reverse Transcriptase-P30 Interaction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Bandy	Sr. Investigator	LMB, NCI
OTHER:	J. Bookout	Staff Fellow	LMB, NCI
	K. Appich	Jr. Investigator	LMB, NCI
	C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)
Dr. Donald C. Fish, Frederick Cancer Research Center

LAB/BRANCH
Laboratory of Molecular Biology

SECTION

NCI, NIH, Baltimore, Maryland 21211
INSTITUTE AND LOCATION

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.4	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Reverse transcriptase-p30 complex was detected in the thymocytes and spleenocytes of AKR mice prior to the onset of disease. The amount of the complex is maximum when the clinical symptoms manifest. In order to inhibit such complex formation, several treatment of AKR mice, e.g., injecting polyA:U, interferon are now being conducted.

PHS-6040
(Rev. 10-76)

Project Description:

Since both p30 and reverse transcriptase are present in the virion core and they stimulate DNA synthesis, it was postulated that they remain in the organized molecular structure and efficiently synthesize DNA. This coupled with the actual isolation of reverse transcriptase-p30 complex from the virion itself suggested to us that the formation of the complex may be an important pre-requisite of the assembly of the virus or for activating the oncogenicity of the virus. In this connection, a reverse transcriptase-p30 complex was isolated from the sub-cellular fractions of thymocytes and spleenocytes of AKR/J mice (which develop spontaneous leukemia at 28 weeks of age) strating at 15 to 20 weeks of age. Such complex formation is maximal when the clinical symptoms becomes manifest. Interestingly, the complex is present only in the target organs, e.g., thymus and spleen whereas non-target organs are devoid of such complex. However, the animals treated with antiviral IgG which delayed the development of spontaneous leukemia, the complex formation appear much later in life, but prior to the development of disease.

Publications:

Bandyopadhyay, A.K. and Levy, C.C., 1978. Existence of reverse transcriptase-p30 complex in AKR mice. Proc. to Amer. Assoc. Cancer Res. 19, 220.

Bandyopadhyay, A.K., Fish, D.C. and Levy, C.C., 1978, Existence of reverse transcriptase-p30 complex in AKR mice after a high incidence of spontaneous lymphocytic leukemia. J. Virol. (In preparation).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06981-01 LMB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Search for a Compound Which Will Restrict the Binding Between Reverse Transcriptase P30 Complex

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. K. Bandy	Sr. Investigator	LMB, NCI
OTHER:	C.C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

Dr. Stanford Moore and Dr. Dalton Wang, Rockefeller University

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.5	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A new compound, polyspermine RNase, can bind specifically with Rauscher leukemia virus reverse transcriptase and inhibit DNA synthesis. The effect of such compound in RNA tumor virus replication are now being studied.

Project Description:

If the formation of reverse transcriptase-p30 is the pre-requisite of the assembly of the virus, it is necessary to search for a few compounds which will bind with reverse transcriptase and inhibit the formation of reverse transcriptase-p30 complex. So far, we have found a compound, polyspermine RNase, which binds with reverse transcriptase. At this point, it will be interesting to know whether such a compound can inhibit reverse transcriptase-p30 complexes either in vitro or in vivo.

Publication:

Bandyopadhyay, A.K., Wang, D., Moore, S. and Levy, C.C., 1978. Binding between Rauscher leukemia virus reverse transcriptase and polyspermine RNase. J. Biol. Chem. (In preparation).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06982-01 LMB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Study of the Binding Between Reverse Transcriptase and Hodgkin's Disease Patients' RNase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Bandy	Sr. Investigator	LMB, NCI
OTHER:	C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Rauscher leukemia virus reverse transcriptase can bind specifically with plasma RNase of Hodgkin's disease patients. The relationship of such binding to the development of disease is now being evaluated.

PHS-6040
(Rev. 10-76)

Project Description: Z01 CM 06982-01 LMB

It was demonstrated in this laboratory that Rauscher leukemia virus reverse transcriptase can specifically bind with the plasma RNase of Hodgkin's disease patients. This work was extended to several other reverse transcriptases either from other mouse or cat or primate viruses. RNase from Hodgkin's disease patients is unable to bind with reverse transcriptase either from mouse ecotropic virus AKRtype, or FelV or SSV-1. However, moloney leukemia virus reverse transcriptase can bind with the same RNase to the same extent as Rauscher leukemia virus reverse transcriptase. The binding study between Hodgkin's disease RNase and reverse transcriptase, isolated from human leukemic cells are now being conducted. If such binding is positive, this suggests perhaps a possible role for serum RNase in the development of Hodgkin's disease. Under these circumstances, we are studying currently, the binding ability of plasma RNase to reverse transcriptase at various stages of Hodgkin's disease.

Publication:

Bandyopadhyay, A.K., Levy, C.C. and Mardiney, M.R., 1977. Complexing between Rauscher leukemia virus reverse transcriptase and plasma RNase from Hodgkin's disease patients. J. Biol. Chem. 252, 7783-7787.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06983-01 LMB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Inhibition with Poly(A)-Poly(U) of Endogeneous C-Type Virus Expression in the AKR Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Bookout	Staff Fellow	LMB, NCI
OTHER:	A. Bandy	Sr. Investigator	LMB, NCI
	C.C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

Dr. Robert Friedman, NIH

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.9	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of PolyA:U injections on endogeneous C-type virus replication and expression of leukemia in the AKR mouse is being examined. The polynucleotide complex not only extends the mean life span of the animal but also suppresses virus expression in spleen lymphocytes.

-6040
(Rev. 10-76)

We observed previously that a multiprotein complex of reverse transcriptase bound to p30 stimulated DNA synthesis several fold and that, although virus expression can be detected even before birth, such complexes could not be isolated from the thymus and spleen of AKR mice before 10 weeks prior to the manifestation of leukemia. It has been observed further that polyA:U doses successfully prolong the long term survival of AKR mice. Present studies have shown that polyA:U injections into 11 week old mice cause a 45% and 32% decrease in viral mRNA in spleen lymphocytes at 16 and 45 weeks of age, respectively. Since the number of proviruses endogeneous to the AKR mouse may be several hundred and also since the ability to induce leukemia may be obtained through recombination among these proviruses, the reduction in viral mRNA suggests that the injections of the polynucleotide complex inhibit endogeneous virus replication. This in turn may suppress the recombination necessary to result in the expression of disease. The effects of multiple polyA:U treatments are being examined to determine at what step (mRNA production or enhanced degradation, reverse transcriptase-p30 complex formation, and viral phenotypic or genotypic mixing) inhibition occurs. The effect of interferon and interferon-treated cell extracts of reverse transcriptase-p30 complex formation will also be examined.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06984-01 LMB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Study of Reverse Transcriptase-P30 Interaction in Relation to DNA Synthesis.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.K. Bandy	Sr. Investigator	LMB, NCI
OTHER:	K. Appich	Jr. Investigator	LMB, NCI
	B. Meric	Jr. Investigator	LMB, NCI
	C.C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)
Dr. Ralph Arlinghaus, University of Texas

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.6	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Reverse transcriptase and P30 have been purified from several retroviruses. The intraspecific and interspecific interaction of these two proteins have been studied.

-6040
(Rev. 10-76)

Project Description: Z01 CM 06984-01 LMB

All of the known viral proteins were purified from Rauscher leukemia virus using a single batch of virus. The major internal proteins p30 can specificially bind with reverse transcriptase and stimulate both heteropolymeric and homopolyermic transcription. In order to understand how widespread this phenomenon of complex formation is, these two proteins were isolated from several other type C viruses and were examined for their ability to bind each other. In each case, p30 can bind with its autologous reverse transcriptase and the resultant complexes all have similar physical properties. P30 binds also with heterologous reverse transcriptase and in this case the resultant complex is more readily dissociated much lower than the autologous reverse transcriptase-p30 complex. Furthermore, autologous p30 can replace heterologous p30 from the heterologous reverse transcriptase-p30 complex.

Since the tumor virus core contains several other proteins, e.g., p10, p15, p12, it is felt that the efficiency of DNA synthesis can be increased several fold by interacting other viral proteins with reverse transcriptase-p30 complex.

Publications:

Bandyopadhyay, A.K., Appich, K. and Levy, C.C., 1978. Simultaneous purification of viral proteins from Rauscher leukemia virus. Anal. Biochem. (In preparation).

Bandyopadhyay, A.K., 1977. Effect of virus specific proteins on Rauscher leukemia virus reverse transcriptase. J. Biol. Chem. 252, 5883-5887.

Bandyopadhyay, A.K., Mardiney, M.R. and Levy, C.C., 1978. Effect of p30 on heteropolymeric transcription of viral 70S RNA. Archiv. Biochem. Biophys. (In preparation).

Bandyopadhyay, AK. and Levy, C.C., 1978. Effect of virus specific proteins on reverse transcriptase: Intraspecific and interspecific interaction between p30 and reverse transcriptase. J. Biol. Chem. (In press).

Bandyopadhyay, A.K. and Levy, C.C., 1978. Formation of intra-interspecific complex between reverse transcriptase and p30. Proc. to Annual Meeting of American Society of Microbiol. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06985-01 LMB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Polypeptides Involved in Herpes Simplex Virus Replication and Cell Transformation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Bookout	Staff Fellow	LMB, NCI
OTHER:	C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Herpes simplex virus (HSV) induced proteins are being studied to determine their roles in virus replication and cell transformation. Polypeptides have been characterized as to their sequestration within the cell, nucleic acid binding properties, and presence of phosphorylated subcomponents.

Project Description:

Cells infected with HSV produce virus specified polypeptides (about 47 for type I strains and 51 for type II strains). Specificity has been defined using one or more of the following criteria: a) increased rates of synthesis post-infection; b) strain variations in polypeptide properties; and c) immunological specificity. We observed that, through virion assembly is in the nucleus, 10 polypeptides in type I infection and 5 polypeptides in type II infection appear to be found only in the cytoplasm. Nine polypeptides of type I and 20 of type II reside primarily in the nucleus while the remaining either reside in both sites or cannot be presently assigned. Thirteen phosphoproteins are induced in type I infections (9 in type II) of which 54% reside in the cytoplasm; however, only one of the cytoplasmic polypeptides of type II is phosphorylated. A total of 16 type I polypeptides were found to have affinity for nucleic acids and 4 of these polypeptides reside primarily in the cytoplasm. Eleven polypeptides induced after type II infection have similar affinity yet only one remains in the cytoplasm. These findings suggest that many polypeptides of type I, having similar molecular weights and kinetic patterns of synthesis postinfection, also exhibit similar properties of nucleic acid affinity, phosphorylation, and location within the cell. Sequestration of nonstructural polypeptides in the cytoplasm suggests further that some of these polypeptides have regulatory or enzymatic functions and serve to bring about cytoplasmic alterations necessary for virus replication.

Further studies will examine a) the presence of these polypeptides in tissue transformed by HSV, and b) the function of cytoplasmic polypeptides in degradation or stabilization of viral mRNA, in the selective translocation of viral mRNA into the cytoplasm, and in protein synthesis.

Publication:

Bookout, J.B. and Levy, C.C. Properties of Herpes simplex virus-specified polypeptides involved in virus replication. Manuscript in preparation, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06986-01 LMB

PERIOD COVERED
October 1, 1977 to September 31, 1978

TITLE OF PROJECT (80 characters or less)

Control Mechanisms by RNase C Type Virus Replication in the Balb/C Mouse

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Bookout	Staff Fellow	LMB, NCI
OTHER:	A. Bandyopadhyay	Sr. Investigator	LMB, NCI
	C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro and in vivo experimentation has shown that infection with murine sarcoma virus (Mo-MSV (MLV)) resulted in rapid decreases in amounts of cytoplasmic ribonuclease activity which correlated inversely with levels of viral specific cytoplasmic mRNA and with concentrations of reverse transcriptase and p30 associated with the microsomal fractions. The significance of these changes are being studied and infections with murine leukemia virus (mo-MLV) are being examined to see if similar alterations occur.

PHS-6040
(Rev. 10-76)

Project Description: Z01 CM 06986-01 LMB

Cells infected in tissue culture with MSV (MLV) rapidly lost cytoplasmic RNase activity while similar cell lines infected with Rauscher leukemia virus showed little decrease in activity. In addition spleen cells from mice injected with MSV (MLV) showed elevations in levels of p30, reverse transcriptase, and virus specific mRNA which peaked in the cell cytoplasm during the time of maximum fibrosarcoma growth and decreased as the tumors spontaneously regressed. During the period of maximum viral expression in the spleen cells the levels of RNase associated with the polysomes dropped off drastically, then rose again during regression. Our previous observations that serum RNase from Hodgkin's disease patients binds specifically with reverse transcriptase, inhibiting its activity, suggested that somecontrol mechanism was being observed. The present observations suggest further that some form of symergism may exist between the early expression of C type virus in spleen lymphocytes and tumor expression in the animal. Present studies of MLV injections, in which no solid tumor develops, were designed to help explain the synergistic effects observed. Future plans include tests to determine if microsomal RNase will bind to reverse transcriptase and if reverse transcriptase-p30 complex formation is prevented in the presence of RNase.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06987-01 LMB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Mapping of the Promotor Regions and the Polypeptide Products of HSV-1 and HSV-2

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Brown	Staff Fellow	LMB, NCI
OTHER:	N. Biswal	Sr. Investigator	LMB, NCI
	C.C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.25	OTHER: 0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies are designed to: first, elaborate on the molecular biology of herpes simplex virus types 1 and 2 by correlating functions with specific viral coded products and mapping the genes for these products; and, second, lead to more effective probes to investigate the presence of protions of the viral genome in cervical cancer biopsy specimens.

Viral DNA is being replicated and transcribed in vitro in order to map the promotor regions. Extracts from control and HSV infected cells are being investigated to determine the presence of factors that enhance the specificity of the polynucleotide syntheses. Similarly, proteins are being synthesized in vitro from viral coded RNA. The products will be identified by immunological and other procedures. Hybridization of the mRNAs to restriction endonuclease fragments will lead to mapping of the genes for their polypeptide products.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CM 06989-01LMB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Functional Significance of Polyadenylic Acid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Karpetsky	Sr. Investigator	LMB, NCI
OTHER:	C.C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

NCI, NIH, Baltimore, Maryland 21211

INSTITUTE AND LOCATION

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The synthesis of poly(A) tracts having 5, 18, 31, 41, 59, 73, 100 and 150 residues, covalently attached to the 3' termini of E. coli 5S $[^3H]$ rRNA (5S rRNA-$(A)_n$) was carried out with polynucleotide phosphorylase. These polyadenylated RNAs and unmodified 5S rRNA were utilized as substrates for ribonucleases from Citrobacter, Enterobacter, bovine pancreas, human spleen and human plasma. In all cases, short lengths of poly(A) (n=18) induce a powerful inhibition (38-87%) of RNase activity, which increases as the poly(A) tract is lengthened. The poly(A) mediated protection of substrate from degradation extends to RNA molecules which are not covalently linked to poly(A), demonstrating that a sub-population of poly(A) containing RNA molecules can contribute to the stability of other RNAs by inhibiting the RNase mediated hydrolysis of these substrates. The structure of 5S rRNA (T_m=61°C) is altered upon covalent attachment of poly(A) (T_m=46°C if 31 adenylic acid residues are present at the 3' termini). Thus, under certain conditions, 5S rRNA $(A)_n$ behaves quite differently from a mixture of 5S rRNA and poly(A). Covalent attachment of poly(A) results in a better inhibitor of RNase activity than free poly(A)[184]. Furthermore, if inhibition is reversed, 5S rRNA$\cdot(A)_n$ is hydrolyzed more rapidly by RNase than 5S rRNA+poly(A).

Preliminary results indicated that polyadenylic acid ((A_n)) inhibits the activity of pyrimidine specific ribonucleases (RNases). Because many messenger RNAs contain $(A)_n$ at their 3' termini, a model system designed to aid in the study of this inhibition was constructed in the following manner. Polynucleotide phosphorylase was used to prepare poly(A) tracts, having 5, 18, 31, 41, 59, 73, 100 and 150 residues, covalently attached to the 3' termini of E. coli 5S $[^3H]$ rRNA (5S rRNA-$(A)_n$). Ribonucleases, differing in their pyrimidine specificity, were purified to homogeneity from the following sources: Citrobacter sp., Enterobacter sp., human spleen, and human plasma. Bovine pancreatic RNase was purchased. The polyadenylated RNAs and unmodified 5S rRNA were utilized as substrates for these enzymes. In all cases, short lengths of poly(A) (n=18) induce a powerful inhibition (38-87%) of RNase activity. Furthermore, inhibition increases as the poly(A) tract is lengthened. The poly(A) mediated protection of substrate from degradation extends to RNA molecules which are not covalently linked to poly(A). Thus, the rate of hydrolysis of nonpolyadenylated substrates such as yeast RNA or poly(C) decreases upon the addition of 5S rRNA-$(A)_n$ to the reaction mixture. This finding demonstrates that a sub-population of poly(A) containing RNA molecules can contribute to the stability of other RNAs by inhibiting the RNase mediated hydrolysis of these substrates. Covalent attachment of $(A)_n$ induces certain marked changes in the physical parameters of 5S $[^3H]$ rRNA. Examination of the changes in absorbance (260nm) that occur as samples of 5S $[^3H]$ rRNA-$(A)_n$ are heated from 24°-96°C revealed that the structure of the 5S rRNA molecule is altered upon addition of 3' terminal segments of $(A)_n$. For example, T_m, ΔH^o, and ΔS^o (the enthalpy and entropy, respectively, for the thermal transition) for 5S $[^3H]$ rRNA are 61°C, kcal and 101 cal/°C while for 5S $[^3H]$ rRNA $(A)_{31}$ these quantities are 46°, kcal and cal/°C. As the $(A)_n$ chain length is increased, both ΔH^o and ΔS^o decrease linearly -- thus a net disordering of the 5S rRNA molecule occurs as a result of the addition of 3' terminal segments of $(A)_n$. Thermodynamic constants obtained from heating solutions of 5S rRNA and $(A)_n$ indicated that little, if any, change in the structure of 5S rRNA is induced by the presence of non-covalently bound $(A)_n$. The results from these purely physical chemical studies prompted us to make a comparison between results from experiments in which RNases are treated with either 5S $[^3H]$ rRNA $(A)_n$ or 5S $[^3H]$ rRNA+$(A)_n$, because the RNA or $(A)_n$ segments of 5S rRNA $(A)_n$ may be recognized and treated by the enzyme quite differently from either 5S rRNA or $(A)_n$ alone. Using RNases from either bovine pancreas or human spleen, it was found that covalent attachment of $(A)_n$ to the substrate molecule (5S $[^3H]$ rRNA) produced an inhibitor superior to free $(A)_n$. The ratio of K_i's ($(A)_n$ attached/$(A)_n$ free), for example, was 0.5 using the human spleen RNase. Additionally, under certain circumstances, the substrate portion of 5S $[^3H]$ rRNA $(A)_n$ behaves quite differently from 5S $[^3H]$ rRNA as the following example demonstrates. Increasing the ionic strength of a mixture containing human plasma RNase and either 5S $[^3H]$ rRNA·$(A)_n$ or 5S $[^3H]$ rRNA+$(A)_n$ induces a reversal of inhibition in either case. Enzyme activity for 5S $[^3H]$ rRNA+$(A)_n$ never exceeds that for 5S $[^3H]$ rRNA alone, i.e., the fractional activity is always $\leq$ 1.0, regardless of the ionic strength. However, using 5S $[^3H]$ rRNA·$(A)_n$, fractional activities of $>$ 1.5 are obtained at moderate ionic strengths. These results suggest that in the absence of inhibition by $(A)_n$, the substrate portion of 5S $[^3H]$ rRNA·$(A)_n$ is more susceptible to hydrolysis by this enzyme than is 5S $[^3H]$ rRNA alone. Thus, conclusions from biochemical studies mesh well with the results of physical studies. The structure of 5S rRNA is altered by the addition of 3'-terminal $(A)_n$ segments and, using certain RNases, the RNA portion of the molecule is more susceptible to hydrolysis under conditions in which inhibition is reversed. Furthermore, covalently attached $(A)_n$ is a better inhibitor of RNase activity than free $(A)_n$. These observations would seem to have important implications for the stability of messenger RNA - since many mRNA molecules have 3'-terminal segments of $(A)_n$. A functional role for the $(A)_n$ segment is clearly suggested - namely inhibition of RNase activity with concomitant protection of the heterogeneous portion of the moelcule. The rate of total RNA hydrolysis may be easily regulated by the length of the $(A)_n$ segments. Also, all RNA molecules in solution are protected from RNase mediated hydrolysis by the presence of $(A)_n$ tracts in a portion of total RNA. Our results also suggest that, although covalently linked $(A)_n$ tracts protect all RNA molecules by a specific interaction with pyrimidine specific RNase, these segments also induce a specific structural change in the RNA molecule to which they are linked - indicating that $(A)_n$ containing mRNA may differ from mRNA lacking $(A)_n$ by more than simply the presence of polyadenylic acid. Studies are currently underway to determine what portions of the substrate molecule are altered by the presence of 3'-terminal $(A)_n$ tracts, and to define which factors, present within the cell, may regulate the inhibition of RNase activity induced by $(A)_n$.

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SUMMARY REPORT
OFFICE OF THE ASSOCIATE DIRECTOR
CANCER THERAPY EVALUATION PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

The Cancer Therapy Evaluation Program (CTEP) performs the following functions within the Division of Cancer Treatment: (1) Plans, evaluates, and coordinates extramural clinical research programs testing combined modality approaches and the testing of investigational new agents. (2) Directs the evaluation of the effectiveness of specific types and methods and agents in the clinical treatment of cancer. (3) Coordinates with the Office of the Director, DCT, the exchange of scientific information with foreign organizations and institutions on all aspects of cancer treatment. (4) Coordinates the activities of the branches within the Program to assure orderly evaluation and assessment of drug compounds and of other specific modalities in treating human malignancies. (5) Assesses the activites of the Program as a whole and advises the Director, DCT as to new areas of promising potential in the clinical treatment of cancer in humans.

I. Organizational Changes

During 1977-78, the Combined Modality Branch and the Clinical Investigations Branch (CIB) were merged into one branch with the latter designation, under the direction of Dr. Hugh Davis. The merger was effected because of the opportunity to bring the two Branches within one location (Landow Building) and to facilitate scientific exchange along disease oriented lines. The Investigational Drug Branch (IDB), which remained in Building 37 together with the Office of the Associate Director, was reorganized into two sections: Drug Regulatory Affairs and Drug Evaluation and Reporting Sections. The CIB, now under the direction of Dr. Raymond Weiss, will be constituted into two sections: A Medicine Section and a Surgery and Nutritution Section, acquiring some grant-supported responsibilites in respective areas. In addition, changes are anticipated which will be fully activated by October, 1978, resulting in the creation of a Radiotherapy Development Branch and a Clinical Projects Branch. These new Branches are a result of transfer of grant programs from The Division of Cancer Research, Resources, and Centers. These include all those related to radiobiology and radiotherapy, including high LET, and those related to multifaceted projects targeted towards development of new therapies in specific conditions, respectively. These new Branches wlll interact closely with the CIB in clinical trials, and with the IDB and the Developmental Therapeutics Program in the area of drug development. These Branches will be physically located next to the CIB in the Landow Building.

In July, 1977, James G. Schwade, M.D., a board certified radiation oncologist, joined the CTEP as Special Assistant for Radiation Oncology. In addition to serving as CIB monitor for the Radiation Therapy Oncology Group, he has coordinated CIB activities dealing with radiation therapy in the other clinical trials cooperative groups, as well as in contract studies. His efforts have also gone toward the organization of an esophageal cancer study group, currently in the process of being formed through the involvement of a number of U.S. and foreign centers with unusually large volumes of this diesase.

Dr. Schwade's major efforts in the CTEP have been directed toward the organization of clinical evaluation of radiation sensitizing and protecting compounds, as well as development of a synthesis, screening, and toxicologic program for radiation sensitizing and protecting drugs in conjunction with Dr. Daniel Rubin, Special Assistant for Scientific Coordination in the Office of the Director, DCT. This has resulted in the conduct of a Phase I clinical trial of the hypoxic cell radiosensitizer, misonidazole (Ro-07-0582) under the auspices of the Radiation Therapy Oncology Group (RTOG) at the University of California, San Francisco, and Roswell Park Memorial Institute. Ten additional Phase II protocols, to be conducted by the RTOG, and a Phase II-3 protocol to be conducted by the Brain Tumor Study Group (BTSG) have been developed with Dr. Schwade's assistance and are currently ready for activation. To facilitate communication among the various areas of the DCT concerned with radiation sensitizer and protector development, an intramural committee, drawn from various programs within the DCT, has been formed, chaired by Dr. Schwade, who also functions as the DCT Radiosensitizer/Radioprotector Analog Committee Chairman. Along with Dr. John Penta, Dr. Schwade serves as an NCI representative to the Radiation Oncology Subcommittee Radiation Sensitizer/Radioprotector Working Group, a committee of extramural scientists assembled to advise the DCT in radiation sensitizer and radioprotector development. In conjunction with members of this group, Dr. Schwade has helped to adapt the DCT Treatment Linear Array to accomodate the radiation sensitizing and protecting agents along the DCT Decision Network. The long-range goal of these efforts is the integration of the development of radiation sensitizing and radiation protecting compounds into the on-going drug development program in the DCT.

In addition, Dr. Schwade has been involved with the NCI/PAHO Collaborative Treatment Project, organizing the integration of radiation therapy related projects into this program.

II. Office Activities

The Office of the Associate Director (OAD) is responsible for integrating the efforts of program branches. In addition, it assists the Branches in setting up activities such as conferences, when such assistance is needed. Several responsibilities, primarily of the office are reviewed here, and changes from last year's Annual Report are noted as follows:

1. Data Center for DCT Supported Clinical Trials.

This function has been transferred to the CIB in the Landow Building. The Program Coordinator, Elise Mackie, supervises the files of active clinical trials, as well as assembling, analyzing, and providing information on clinical trials in response to various NIH and NCI requests. In addition, an ongoing function is that of supplying activated protocols to the International Cancer Research Data Bank (ICRDB, see 1977 Report). Intramural protocol studies are also logged in the file.

2. Coordination and Activation of Clinical Trials.

The IDB activates all Phase I protocols and the CIB protocols dealing with Phase II, pilot studies, and disease-oriented comparative trials which are under direct contract or grant support. The OAD is responsible for the activation of protocols from international agreements after review by respective branches, CIB or IDB. Protocol studies are ongoing with French institutions under the U.S. - France Agreement (Appendix I) and are planned with various Latin American Cancer Centers under the NCI-PAHO Research Program (Appendix II). In addition to these protocol studies, the OAD is responsible for coordinating review and activation of protocols developed under the Veterans Administration Interagency Agreement, the Georgetown Gastrointestinal Cancer Research Contract (CM-67110), the NCI-Milano Contract (NO1-CM-33714), and the WHO Melanoma Contract (NO1-CM-43726). These functions will eventually be transferred to the CIB as has been done with other contracts now that the CIB is more fully staffed.

3. Program Review and Budgetary Allocation.

All contracts had annual review by either the Combined Modality Committee, Clinical Trials Committee, or by an intramural review committee. These activities are summarized by the annual reports of the Executive Secretaries, Drs. Kisner and Henney, for the chartered committees and by Dr. Weiss for the intramural review committee.

A VA-NCI Review Body was set up to review the activities of the Veterans Administration Surgical Adjuvant Group (VASAG) and the Veterans Administration Lung Group (VALG) and this body met for the first time on May 2, 1978. Full minutes are under preparation following mail ballot of some recommendations.

Contract budgets are negotiated annually upon recommendations of appropriate review committees. The cooperative group grant allocations are made following review by the Cancer Clinical Investigations Review Committee (CCIRC), approval by the National Cancer Advisory Board (NCAB), and budgetary possibilities to fund fully or partially according to priority scores. These decisions are made by the Director, DCT following the recommendations made at a meeting of the AD, Program Administrative Officer, Chief CIB, and the Program Director. Budgetary guidelines for the year are developed in meetings of the Branch Chiefs and the AD, and eventually presented to the Director and the DCT Program Staff.

4. Review and Development of Therapeutic Strategies.

An ongoing review of various aspects of clinical trials has been a valuable program function, with important feedback into other DCT programs. This includes participation of CTE personnel in analog committees (Muggia and Rozencweig), review of toxicology data and correlation with human tolerance of anticancer drugs (Penta and Rozencweig), standardization of data reporting (Bono, McGuire, Muggia, Rozencweig, Lee) - which is being achieved for Phase II contracts -, review of adjuvant trials (Louie, Muggia, Rozencweig), review of immunotherapy trials (Mikulski), possibilities of steroid hormones coupled with cytotoxic moieties (Muggia), and correlations of animal data with antitumor activity in man (Rozencweig) together with the Drug Evaluation Branch and A.D. Little. These projects are mentioned because they represented special efforts during the year. Other specific projects which may be recognized from meetings and publications included participation in: review of usefulness of antitumor antibiotics (Bono, Davis, Rozencweig, Slavik and Muggia), review of osteosarcoma trials (Louie and Muggia), review of head and neck cancer therapy (Browder, Schwade, Rozencweig, Muggia, Turrisi), the role of amino acid depletion in cancer therapy (Muggia), future directions for high dose methotrexate studies (Bono and IDB Staff), review of hexitol therapy (Chiuten, Rozencweig, Muggia).

These activities listed are those extending beyond the usual function of the Branches in developing disease-oriented stragegy and in monitoring clinical trials with new drugs.

5. Staff Meetings, Orientation, and Training.

During the first few months staff meetings include an orientation program (Appendix III). These are particularly designed for members joining the Program generally as staff associates with the IDB, and for individuals who have not participated in the CTE activities previously. These, coupled with conferences of the COP, constitute a thorough coverage of clinical oncology and clinical trials methodology.

Other functions of the staff meetings include: review of recent, past, and future activities, discussion of adverse drug reactions, administrative announcements, and review of highlights within Branch programs.

Guest workers in 1977-1978 in the CTEP included Louise Lev and Shoshana Biran (both from Israel) for three months and two weeks, respectively, Mohamed Reda Hamza (Egypt) for one month, and Maurice Staquet (Belgium) for three months.

III. Progress Report of Program Activities.

Some of the activities of the OAD were summarized in the preceding section. Here we summarize activities of various program areas, which are included in greater detail within the reports of each Branch. The following are particularly relevant to the Program:

1) Meetings Including Program Participation.

These are listed in Section VIII, and represent those meetings which were sponsored by the CTEP or in which we took an active role in joint sponsorship with other DCT Programs, or sponsored by our contractors or grantees. These represent in great part, "Consensus Development" or "State of the Art" meetings and in others, "Think Tank" type sessions.

2) Sponsorship of Drug Studies.

To date, we have 102 active IND's of which 15 are for commercially active drugs. In 1977, seven new IND's were submitted to the FDA and approved. In 1978, L-Alanosine was submitted and approved. These drugs included:

Levamisole (NSC 177023)
Misonidazole (NSC 261037)
Bruceantin (NSC 165563)
AMSA (NSC 249942)
Pyrazolo-Imidazole (NSC 51143)
Indicine-N-Oxide (NSC 132319)
PALA (NSC 224131)

It should be noted that the PALA IND was obtained with waiver of the 30-day notice because of unusual interest in early activation of protocols.

Seven additional IND's are currently in preparation including:

PCNU
Pentamethylmelamine

DON
5-Methyltetrahydrohomofolate
Soluble ICRF-159
Heroin
Tetrahydrocannabinol

This represents an activity similar to the preceding year when Phase I studies were initiated on chlorozotocin, deazuridine, rubidazone, hycanthone, aminothiadiazole, maytansine, dichloroallyl lawsone, neocarzinostatin, anguidine, and gallium nitrate, some akyl lawsone, neocarzinostatin, anguidine, and gallium nitrate, some begun on already existing IND's.

Currently there are 18 drugs classified as Group A, of which 8 have passed Decision Network Four and are, therefore, in Phase II trial. Group B consists of 62 drugs, and Group C, 7. One drug in Group C, L-asparaginase (E. Coli), became commercially available (Elspar-Merck) in April, 1978, but was replaced by L-asparaginase (Erwinia) on the Group C list. Immunomodulators (i.e., Levamisole) or drugs used in supportive care (such as heroin) bypass the Group A designation and enter Group B, with or without restrictions in regards to the type of studies that are acceptable.

Protocol activity in new drugs which were activated this year is the following:

Drug	# of Protocols	Estimated # of Patients
Levamisole	21	could not estimate
Misonidazole	3	35
Bruceantin	2	50
AMSA	6	85
Pyrazolo-Imidazole	5	30
Indicine-N-Oxide	3	10
PALA	6	35
TOTAL	46	245 + Levamisole protocols

3. Clinical Trial Statistics.

a. Overall Resources.

The CTEP supports under grant, contract, or interagency agreement clinical trials dealing with cancer treatment performed in institutions throughout the United States and some abroad. The following is the number per mechanism:

Number of institutions in cooperative groups - 685
Number of contractors in DCT clinical trials - 72
Number of institutions in VA groups - 50

This network of groups has an extensive geographic distribution, which was detailed in an analysis performed by the Program Analyst (Ms. Elise Mackie, Landow 8C03, 496-6056) for the Director, DCT. Some overlap exists of multigroup representation within one institution and also contract and group-supported activities within one center. An additional analysis was performed by Ms. Mackie on the extent of clinical trials resources currently available to the Cancer Centers Program. Both these reports performed during the early part of 1978 are available on request. In addition a compendium of clinical investigators, personnel in group headquarters, and other organizations such as statistical offices dealing with clinical trials activities was assembled and published during 1978.

b. Protocol Studies

Phase I studies are performed by contract under the aegis of the IDB. The RTOG, however, was involved in a Phase I study of misonidazole, a hypoxic radiosensitizer, and six other special studies dealing with radiotherapy development and registry of complications. Two studies by the Cancer and Leukemia Group B (CALGB), three by the Southeastern Cancer Study Group (SEG), six by the Southwest Oncology Group (SWOG), and eight by the Children's Cancer Study Group (CCG) primarily dealt with drug evaluation. Special studies (i.e., immune evaluation, registries, etc.) by other cooperative groups and contracts numbered nineteen. The balance of the remaining studies up to 484 dealt with disease-oriented activities within the cooperative groups or contracts. A summary of disease-oriented activity by mechanism appears in Table 1. A large number of disease-oriented activities supported by contract are in fact related to Phase II studies with new agents by Phase II/III contractors. In addition, other drug related (Group B drugs) studies are being conducted directly by the IDB via the Cancer Center and New Drug Studies Mechanisms.

c. Patients Entered.

The number of patients entered in Phase I contract-supported studies was enumerated earlier. The entry of patients into the protocols in Table 1 has been obtained from cooperative group headquarters and from reports to project officers. It should be noted that some overlap exists, with the same patient appearing as a separate entry in the same study if a crossover is included, or being entered in more than one study. The same is applicable to some Phase I studies (Tables 2 and 3).

IV. Highlights of Program Activities

1. Organizational

Special accomplishments of an organizational nature which should result in impact on activities of the Program in subsequent years are listed below:

- IDB eliminated a backlog of incomplete annual reports and closed a number of inactive IND's.

- A drug distribution system was worked out in discussions with the FDA which virtually eliminated the category of "independent" investigators while ensuring availability of drugs shown effective to qualified physicians.

- A document of Operational Procedures for Clinical Trials, to be a part of a master file with the FDA was finalized.

- A geographic cooperative group, the Northern California Oncology Group (NCOG) was funded and initiated protocol studies. Protocol studies were also reviewed for another geographic group, the North Central Cancer Group (NCCG).

- Hypoxic radiosensitizer trials were begun within the Radiation Therapy Oncology Group (RTOG).

- Lung cancer adjuvant trials were begun by the newly formed contract-supported Lung Cancer Study Group. More than 100 patients with resected lung cancer were entered in a protocol of intrapleural BCG versus placebo.

- Protocols were being finalized for newly implemented studies dealing with adjuvant head and neck trials (contract-supported via newly formed group), adjuvant testicular cancer trials (intergroup and Phase II/III Study) and colorectal cancer (contract-supported via National Surgical Adjuvant Breast Project, NSABP).

- In collaboration with the Office of International Affairs of NCI and the Pan American Health Organization (PAHO) clinical trials have been worked out between five U.S. cancer centers and eight Latin American institutes in five countries. The program emphasizes clinical trials methodology and exchange of information between these centers and NCI on treatment research.

- New drug studies were initiated in collaboration with the Cairo Cancer Institute for the treatment of bladder cancer in Egypt. Hexamethylmelamine was found to be an effective drug against this bilharzial bladder cancer (Nazli et al, Cancer Treatment Reports, to be published).

- Efficient compilation of IND's and close collaboration with the Developmental Therapeutics Program (DTP) has led to introduction of seven new drugs into clinical trial in 1977.

- The NSABP is continuing to accrue patients into a much needed study of segmental mastectomy, same plus radiotherapy and a total mastectomy in breast cancer.

2. Scientific.

Studies supported by the CTEP appear to be particularly promising or already eventuated in major landmarks of cancer treatment. Among them are the following:

- In a series of studies at Memorial Sloan-Kettering Cancer Center, Golbey and coworkers established the curative potential of combinations containing cis-platinum in advanced testicular cancer (VAB regimens).
- Analogous studies by the SWOG indicated in multiinstitutional studies the effectiveness of a similar regimen: Velban, lolcomycin, and cis-platinum (VBP).
- Studies by the SWOG and Stanford University as part of NCOG indicated that cis-platinum was effective in head and neck cancer as a single agent, and complemented studies by Memorial investigators under contract which indicated the value of cis-platinum - bleomycin combinations given prior to definitive local therapy in this disease.
- In small cell carcinoma of the lung, a large group study by the SWOG indicated a definite percentage of long term (greater than two years) survivors by the combination of cytoxan, vincristine, and adriamycin, followed by radiotherapy (CHOR combination, Livingston, et al, Annals of Intern. Med., January, 1978).
- In other usually chemoresistant cell types of lung cancer, investigators at the Mayo Clinic described more than 1/3 responses with combinations of cytoxan, adriamycin and cis-platinum (CAP) with improved survival.
- The ECOG in analogous, advanced disease studies indicated similar results with one of seven combinations tested: hexamethylmelamine, adriamycin, methotrexate initially piloted at Albany led to best results in percent response rates and survival.
- SWOG studies in Hodgkin's disease indicate that the addition of chemotherapy in Stages I or II definitely lowers disease-free survival, and in longer followup, will investigate effect on overall survival, particularly in subsets prone to relapse such as those with mediastinal involvement.
- A four year update of breast adjuvant studies by Bonadonna in Milan indicate significant improvement in survival in patients with Stage II disease receiving adjuvant chemotherapy with CMF; this difference is predominantly contributed by premenopausal women.

- Sequential breast adjuvant studies by the NSABP, chaired by Dr. Bernard Fisher, continue to show similar effects for L-PAM, and presently no significant difference for combined drug regimens versus L-PAM alone.

- Combined modality studies in Wilms Tumor, Ewing's and rhabdomyosarcoma by the various cooperative groups indicate unequivocal improvements in survival relative to earlier series.

- A search for agents effective against colon cancer led to testing of glutamine antagonists against human tumor xenografts. Diczo-oxo-norleucine (DON), a drug that received brief trial in the 1950's, was identified to have antitumor activity against colon, lung, and breast xenografts and is being readied for clinical trial.

- Several cis-platinum containing combinations within cooperative groups and institutions have indicated efficacy in ovarian cancer, which are being exploited in new group studies.

- Adriamycin and hexamethylmelamine represent two additional agents with efficacy against ovarian cancer, even after relapse from alkylating agents; these have been identified in various group studies of the Gynecologic Oncology Group (GOG), Eastern Cooperative Oncology Group (ECOG), SWOG, and NCOG.

- Studies by the Gastrointestinal Tumor Study Group (GITSG) indicated superiority of 5FU - MeCCNU over the same plus radiotherapy in gastric cancer and 5FU + radiotherapy over radiotherapy alone in locally inoperable pancreatic cancer.

- A nitrosourea with possibly lesser bone marrow toxicity, chlorozotocin, has completed Phase I trial and is being advanced for Phase II trials retaining preliminary activity in colorectal carcinoma and other tumors.

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63. Rozencweig, M., Von Hoff, D.D., Penta, J.S., Catane, R., Louie, A.C., and Muggia, F.M.: Current studies of high dose methotrexate (HDMTX) in the United States. Chemoterapia Oncologica (in press).

64. Staquet, M., Rozencweig, M., and Muggia, F.M.: Predictive value of clinical trial results. Cancer Chemother. Pharmacol. (in press).

65. Turrisi, A.T. III, Rozencweig, M., Von Hoff, D.D., and Muggia, F.M.: The role of bleomycin in the treatment of advanced head and neck cancer. IN: Proceed. of the Symposium on the Bleomycins, Carter, S.K. (Ed.) (in press).

66. Venditti, J.M., Goldin, A., Miller, I., and Rozencweig, M.: Experimental models for antitumor testing in current use by the National Cancer Institute, USA; statistical analysis and methods for selecting agents for clinical trials. IN: Proceed. of the International Symposium of Princess Takamatsu Cancer Research Fund (in press).

67. Von Hoff, D.D. and Rozencweig, M.: Cis-diamminedichloroplatinum (II): A metal complex with significant anticancer activity. Adv. Pharmacol. Chemother. (in press).

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70. Von Hoff, D.D., Rozencweig, M., and Muggia, F.M.: Cytembena: An antitumor drug with a unique structure. Biomedicine 26: 388-392, 1977.

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VI. PRESENTATIONS

Franco M. Muggia, M.D.:

1. Status of GI studies to the Gastrointestinal Cancer Study Group in Rochester, Minnesota, September, 1977.

2. Status of studies in Milan to the Breast Cancer Study Group, September 12, 1977.

3. Orientation lecture for the Clinical Oncology Program, October, 18, 1977.

4. Review of current adjuvant trials in GI cancer to the EORTC Session of the International Conference on Gastrointestinal Cancer, Tel Aviv, Israel, November, 1977.

5. Testicular cancer and cis-platinum to the VIIIth International Symposium of the Princess Takamatsu Cancer Research Fund, November, 1977.

6. Review of clinical trials methods to the Seminario Internacionale de Oncologia Clinica, Torremolinos, Spain, February, 1978.

8. Non-Hodgkin's lymphoma to the Seminario Internacionale de Oncologica Clinica, Torremolinos, Spain, February, 1978.

9. Presentation of a one-day Pre Congress Course to the Vth Cuban Oncology Congress in Havana, Cuba, March 20, 1978.

10. Cancer Chemotherapy to the Albany Regional Cancer Center, Albany, New York, May 10, 1978.

11. Priorities for studies in lung cancer to the World Conference on Lung Cancer Hilton Head, South Carolina, May 13, 1978.

12. Introductory remarks to the CML - Blast Crisis Meeting, Bethesda, Maryland, April 26, 1978.

13. Introductory remarks to the Amino Acid Analog Workshop, Bethesda, Maryland, May 23, 1978.

14. Chemotherapy of head and neck cancer to the University of Maryland's Third Annual Symposium on Advances in Head and Neck Cancer, Baltimore, Maryland, May 18, 1978.

15. Phase II trials with a new drug to U.S. - Japan Cooperative Cancer Program - Joint Meeting on Phase I and II in Cancer Chemotherapy, Tokyo, Japan, June, 1978.

16. New drugs in clinical trial to U.S. - Japan Cooperative Cancer Program - Joint Meeting on Phase I and II in Cancer Chemotherapy, Tokyo, Japan, June, 1978.

Marcel Rozencweig, M.D.:

1. Platinum coordination complexes in cancer chemotherapy. Xth International Congress of Chemotherapy, Zurich, Switzerland, September, 1977.

2. Heavy metals (Chairman). Xth International Congress of Chemotherapy. Zurich, Switzerland, September, 1977.

3. Experience with dibromodulcitol and dianhydrogalactitol in the USA. First International Symposium on Cytostatic Galactitol Derivates, Budapest, Hungary, September, 1977.

4. Clinical experience with dianhydrogalactitol (Chairman). First International Symposium on Cytostatic Galactitol Derivates. Budapest, Hungary, September, 1977.

5. Recent developments with anthracycline antibiotics. Roundtable Discussion on Anthracycline Antibiotics, Rome, Italy, September, 1977.

6. Clinical application of new anticancer drugs and perspectives. Simposio Internazionale di Cancerologia, Cagliari, Italy, September, 1977.

7. Bleomycin in squamous cell head and neck carcinoma. Symposium on the Bleomycins - Current Status and New Developments, Oakland, California, October, 1977.

8. New drugs for early clinical trials. EORTC Early Clinical Trials Group, Brussels, Belgium, November, 1977.

9. Prognostic factors in breast cancer. International Seminar of Clinical Oncology, Torremolinos, Spain, February, 1978.

10. Prognostic factors in Hodgkin's disease. International Seminar of Clinical Oncology, Torremolinos, Spain, February, 1978.

11. Coordination of clinical trials by the Cancer Therapy Evaluation Program of NCI. International Seminar of Clinical Oncology, Torremolinos, Spain, February, 1978.

12. Adjuvant therapy in the USA. Workshop on adjuvant treatments, Berlin, East Germany, February, 1978.

13. Adjuvant chemotherapy - Prospects. Workshop on adjuvant treatments, Berlin, East Germany, February, 1978.

14. New agents for the treatment of lung cancer. World Conference on Lung Cancer, Hilton Head, South Carolina, May, 1978.

15. New agents in cancer chemotherapy. U.S. - Japan Cooperative Cancer Program - Joint Meeting on Phase I and II in Cancer Chemotherapy, Tokyo, Japan, June, 1978.

16. Phase I clinical trials. U.S. - Japan Cooperative Cancer Program - Joint Meeting on Phase I and II in Cancer Chemotherapy, Tokyo, June, 1978.

17. Prognostic factors in breast cancer. International Meeting on Breast Cancer. Athens, Greece, June, 1978.

VIII. CONFERENCES

1. Breast Cancer, 9/12/77
2. Anthracycline Workshop, 9/19/77
3. Ovarian Cancer Study Group, 9/26/77
4. Soft Tissue Sarcoma, 10/17/77
5. Statisticians Meeting, 10/21/77
6. Head and Neck Protocol Writing Committee, 1/27/78
7. Phase I Working Group, 1/19-20/78
8. Sarcoma (followup), 3/17/78
9. High-dose Methotrexate, 3/21/78
10. New Drug Liaison, 3/22/78
11. Pediatric Oncology, 4/1/78
12. Aclacinomycin Workshop, 4/19/78
13. CML - Blast Crisis, 4/26/78
14. Phase I Working Group, 5/15-16/78
15. Ovarian Cancer Study Group, 5/19/78
16. Amino Acid Analog Workshop, 5/23/78
17. Testicular Protocol Writing Committee, 5/26/78
18. Pathologists Working Group, 6/15-16/78
19. Myeloma Treatment Working Group, 7/12/78
20. New Drug Liaison, 9/20/78
21. NCI - EORTC Symposium on New Drugs in Cancer Therapy Brussels, Belgium, 9/7-8/78
22. Cis-platinum and Testicular Symposium, 9/21-22/78
23. Twice Annual Meetings of the Clinical Cooperative Groups - ECOG, SEG, SWOG, CALGB, NCOG, NSABP, PVSG, GITSG, LCSG, CCSG

IX. FOREIGN VISITORS

1. Dr. Ogawa - Tokyo, Japan - 9/13/77
2. Dr. Moyano - Madrid, Spain - 9/15/77
3. Dr. Korec - Yugoslavia - 9/15/77
4. Dr. Gregor - Royal Marsden Institute, London, England - 9/29/77
5. Dr. de Maar - WHO, Geneva, Switzerland - 9/30/77
6. Dr. Said - Pakistan - 10/5/77
7. Dr. Tsukagoshi - Tokyo, Japan - 10/12/78
8. Dr. Galvez - Buenos Aires, Argentina - 10/13/77
9. Drs. Jolles and Simon - France - 10/19/77
10. Dr. Staquet - Jules Bordet Institute, Brussels, Belgium - 10/20/77
11. Prof. Ficari - Rome, Italy - 10/21-23/77
12. Dr. Band - Edmonton, Canada - 10/23/77
13. Dr. Hildebrand - Jules Bordet Institute, Brussels, Belgiu - 12/13/77
14. Prof. Barbieri - Milan, Italy - 1/30/78

18. Clinical trials with high-dose methotrexate. High-dose Methotrexate International Workshop, Florence, Italy, June, 1978.

19. High-dose methotrexate in osteosarcoma (Cochairman of Roundtable). High-dose Methotrexate International Workshop, Florence, Italy, June, 1978.

VII. COMMITTEE MEMBERSHIP, HONORS, AND ACTIVITIES

Franco M. Muggia, M.D.:

Professor of Medicine, George Washington University
Vice-Chairman, Combined Modality Committee
Member, Decision Network
Member, Cancer Treatment Program Staff
Member, Breast Cancer Task Force
Member, Clinical Research Subpanel
Member, Analog Committees of DCT
Member, VA Review Committee
Member, Operating Committee
Member, Drug Evaluation Committee
NCI Co-coordinator for Chemotherapy, U.S. - Japan Agreement
Chairman, Lung Cancer Program U.S. - U.S.S.R. Agreement
Member, Society of Surgical Oncology
Cooresponding member, Social Security Peruvian Society of Oncology
Coordinator for New Drug Projects, U.S. - France Agreement
Coordinator, NCI - Pan American Health Organization (PAHO) Treatment Research
Member, Planning Committee, American Cancer Society Committee on Cancer Chemotherapy
Honorary member, Spanish Society of Oncology
Professor of Pre-Congress Course of the Vth Cuban Oncology Congress
Chairman, U.S. - France Meeting, Paris, France, December, 1977.
Hematology Attending, Veterans Administration Hospital, Washington, D.C., April, 1978
Participation in George Washington University Oncology Conferences which occur monthly

James G. Schwade, M.D.:

Chairman, DCT Committee, Radiosensitizers - Radiation Protector Analog Committee
NCI Representative, Radiation Oncology Coordinating Sub-Committee (ROCS) - Radiation Protectors Working Group
NCI Representative, ROCS - Council of Radiation Oncology Committee Chairmen

15. Dr. Coates - Australia - 3/17/78
16. Dr. Klastersky - Brussels, Belgium - 3/24/78
17. Drs. Paoletti and Veronesi - Milan, Italy - 3/30/78
18. Drs. Frati and Bonmassar, Rome and Perugia, respectively - 4/12/78
19. Dr. Gratterola, Milan, Italy - 4/20/78
20. Dr. Grigoletto - Ospedale - Civile, Pordenone, Italy - 4/20/78
21. Dr. Fiorentino - Padova, Italy - 4/24/78
22. Dr. Koyama - Japan - 5/15/78

TABLE 1
PROTOCOL STUDIES (1977) - SUMMARY BY DISEASE
(Excludes Drug Oriented Studies)

	# Protocols Groups and VA	# Protocols Contract	Total
Leukemia (Adults)	15	1	16
Leukemia (Children)	23	0	23
Lymphomas (HD)	11	1	12
Lymphomas (nHD & general)	10	2	12
Other Hematologic	20	-	20
Pediatric Solid Tumors	21	-	21
Breast Cancer	23	34	57
CNS	14	5	19
Endocrine	3	-	3
Gastrointestinal:			
Colorectal, SI	14	19	33
Other	17	21	38
GU:			
Bladder, Ureter, Urethra	2	3	5
Kidney, General	2	1	3
Prostate	3	2	5
Testes	3	2	5
Gynecologic:			
Ovary	16	11	27
Uterus	7	0	7
Other	13	1	14
Head and Neck	7	8	15
Lung:			
Small Cell	7	4	11
Other	15	19	34
Melanoma	11	6	17
Sarcoma:			
Bone	4	2	6
Other	7	6	13
TOTAL	268	148	416

TABLE 2 - CTEP CLINICAL TRIALS (COOPERATIVE GROUPS)

GROUP	JAN. 1 - DEC. 31, 1977							
	# PATIENTS ENTERED (GROUP-WIDE STUDIES)	# PATIENTS EVALUABLE	PILOTS # PTS ENTERED	# PATIENTS EVALUABLE	ANCILLARY STUDIES # PTS	REGISTRIES # PTS	# PTS SCREENED	TOTAL # PTS ON TREATMENT STUDIES & IN FOLLOWUP
CALGB	1720	1605	336	336	12	2068	NA	5500
CCSG	1585	1585	159	159	46	682	NA	4336
ECOG	3630	3505	112	112	0	48	NA	7495
SEG	2292	1260**	251	73**	7	NA	NA	5500
SWOG	5810	5203	84	81	918	6812	NA	30272
RTOG	916	884	118	118	107	654	19312	1993
NCOG	8	8	47	47	NA	NA	NA	55
GOG	898	813	182	(150)*	846	3995	NA	1871
NSABP	810	780	NA	NA	50	NA	NA	4125
PVSG	605	605	37	37	0	14	0	642
RHDG	0	0	0	0	0	0	0	371
UORG	208	173	NA	NA	208	NA	407	446
WTSG	229	191	NA	NA	NA	NA	NA	892
VALG	577	500	30	27	45	NA	NA	1046
VASAG	613	505	NA	NA	5250	108	1823	2075
TOTAL	19801	17617	1356	1140	6643	14381	21542	66619

* () = estimate
**patients evaluated

TABLE 3 - CTEP CLINICAL TRIALS (CONTRACT)

CONTRACTOR	JAN. 1 - DEC. 31, 1977							
	# PATIENTS ENTERED (GROUP-WIDE STUDIES)	# PATIENTS EVALUABLE	PILOTS # PTS ENTERED	# PATIENTS EVALUABLE	ANCILLARY STUDIES # PTS	REGISTRIES # PTS	# PTS SCREENED	TOTAL # PTS ON TREATMENT STUDIES & IN FOLLOWUP
BTSG	458	456	NA	NA	NA	NA	NA	832
GITSG	581	526	NA	NA	NA	NA	NA	912
LCSG	138	128	NA	NA	NA	138	NA	126
OSG	136	136	NA	NA	NA	NA	NA	(136)*
BREAST PHASE II:								
RPMI	67	63	NA	NA	NA	NA	NA	79
MAYO	102	–	NA	NA	NA	NA	NA	326
Ph. II GI, MAYO	186	NA	NA	NA	NA	NA	NA	321
BREAST, GI, BRAIN, MILAN	233	226	NA	NA	NA	NA	NA	1500
MELANOMA - MILAN	305	271	NA	NA	NA	NA	NA	1143
GEORGETOWN GI RESEARCH PROGRAM	NA	NA	144	144	42	NA	NA	118
Ph. II/III MAYO	448	(380)	NA	NA	NA	NA	NA	836
MDA	547	455	NA	NA	NA	NA	NA	800
SF	421	302	NA	NA	NA	NA	NA	591
SK	497	396	2	2	NA	NA	NA	325
WSU	484	460	NA	NA	NA	NA	NA	663
Ph. I - TOTAL	245	199	NA	NA	NA	NA	NA	245
GI LEEDS	(42)	(36)	NA	NA	300	NA	250	(125)
TOTAL	4990	4284	146	146	342	138	250	9078

APPENDIX I

PROTOCOL STUDIES WITH FRENCH INSTITUTIONS
U.S. - FRANCE AGREEMENT

New Projects

Study	U.S. Investigator	French Investigator
Osteosarcoma	Jaffe	Lagarde, GETO
New Phase II Studies	Muggia	Israel Boiron Jacquillat Mathe
Hyperalimentation	Muggia (Kisner)	Meyer Serrou
Resistant Breast Carcinoma	Muggia (Henney) Macdonald	Israel
Hyperthermia Radiosensitizer	DeVita (Rubin)	Lagarde

Current Projects

Study	U.S. Investigator	French Investigator
Pharmacology of 5 FU and MTX	Chabner Mihich	Meyer
Nitrosoureas	Schein	Mathe
GI Cancer	Macdonald	Lagarde Boiron
Platinum	Muggia	Hayat
Anthracyclines	Frei	Jacquillat

APPENDIX II - CURRENT PAHO PROTOCOLS AND APPROVAL DATES

ARGENTINA

LYN I ARG 77-01 (10/6/77)	A randomized trial of two different chemotherapeutic regimens, BACOP or C-MOPP, in treatment of advanced diffuse histiocytic lymphoma (Pavlovsky, Woolley)
DS I ARG 77-02 (10/6/77)	Phase II trial of chlorozotocin in leukemias, lymphomas, and other hematologic malignancies (Pavlovsky, Woolley)
HN I ARG 77-04 (1/5/78)	Adjuvant chemotherapy and immunotherapy for squamous cell carcinoma of the esophagus (Olivari, Woolley)
GIP I ARG 77-05 (1/5/78)	Randomized trial of radiation plus 5-Fluorouracil in locally unresectable pancreatic cancer (Estevez, Woolley)
HN I ARG 77-03 (Reviewed 11/29/77)	TO BE REVISED - Adjuvant chemotherapy and immunotherapy for squamous cell carcinoma of the oral cavity, oropharynx and larynx, Stages II, III, and IV (MO)

BRAZIL

BR I BRAZIL 77-01 (10/20/77)	Chemotherapy of disseminated breast cancer comparing FAC with FAC plus tamoxifen (Araujo, Bodey)
HN I BRAZIL 77-02 (1/5/78)	Cis-dichloro-diammineplatinum-II (DDP) or cytoxan combination for advanced head and neck cancer (Araujo, Bodey)
DS I BRAZIL 77-03 (12/19/77)	Cis-platinum and bleomycin in epidermoid carcinoma, with special reference to head and neck, cervix, and penis (Gentil, Burchenal)
HN I BRAZIL 78-01 (In review-5/25/78)	Combination chemotherapy for advanced head and neck cancer (Cabral, Bodey)

CHILE

BR I CHILE 78-01 (1/16/78)	Protocol to study the effectiveness of chemotherapy and hormonal manipulations in women with disseminated breast carcinoma (Arroztoas, Ramirez)

APPENDIX II - CONTINUED

COLOMBIA

ME I COL 77-01 (10/6/77)	Combination chemotherapy in disseminated or recurrent malignant melanoma (Ospina, Henderson)
SAO I COL 77-03 (9/14/77)	A study of adjuvant chemotherapy with adriamycin versus adriamycin plus cis-platinum in osteogenic sarcoma (Ospina, Henderson)

PERU

SAO I PERU (1974 - closed)	Radical amputation and prophylactic radiation to lungs and chemotherapy with adriamycin in patients with resectable osteogenic sarcoma (Caceres, Tejada)
BR I PERU INEN 7401 (1974-To be closed)	Simple mastectomy, combination chemotherapy and control in patients treated with radiotherapy for Stage III adenocarcinoma of the breast (Caceres, Tejada)
SAO I PERU INEN 7703 (12/16/77)	Primary combined treatment for patients with resectable osteogenic sarcoma (Caceres, Tejada)
GYC I PERU INEN 7709 (12/29/77)	Chemotherapy for local and metastatic Stage IV uterine cervix carcinoma and for recurrent Stages II and III uterine cervix carcinoma (Single agent versus combined chemotherapy) (Caceres, Tejada)
BR I PERU INEN 7801 (3/9/78)	Phase II study of procarbazine in metastatic measurable adenocarcinoma of the breast (Caceres, Tejada)
BR I PERU INEN 7804 (To be revised-3/9/78)	Chemotherapy and radiotherapy versus chemotherapy and surgery and postoperative radiotherapy with or without immunotherapy in management of Stage III breast cancer (Caceres, Tejada)

APPENDIX III

SCHEDULE FOR ORIENTATION SESSIONS

July 3	Role and organization of the Cancer Therapy Evaluation Program	Dr. Muggia
July 10	Clinical Projects Branch	Dr. Halterman
July 17	Clinical Investigations Branch	Dr. Weiss
July 24	Investigational Drug Branch	Dr. Bono
July 31 -	Radiotherapy Development Branch	Dr. Mahoney
August 7	Clinical Projects Branch	Dr. Halterman
August 14	Clinical Investigations Branch	Dr. Weiss
August 21	Investigational Drug Branch	Dr. Bono
August 28	Radiotherapy Development Branch	Dr. Mahoney

These meetings are in addition to the regularly scheduled Staff Meetings on Tuesday Mornings and will take place on Monday afternoons from 3:00 - 5:00.

CLINICAL INVESTIGATIONS BRANCH (CIB)

Introduction

The Clinical Investigations Branch (CIB) is responsible for the scientific administration of the national cooperative clinical trials groups (the Cooperative Group Program). It is also responsible for scientific monitoring of drug and disease-oriented contracts.

The Cooperative Group Program began under the auspices of the Cancer Chemotherapy National Service Center (CCNSC) and was primarily created to test the activity of the many new agents emerging through the effort of the NCI drug development program. It provided the majority of the information on activity of new drugs through Phase I and II testing. The program underwent many administrative changes in the ensuing years: CCNSC 1955-66; Chemotherapy 1966-1968; Extramural Activities 1968-1972; Division of Cancer Research, Resources and Centers 1972-75.

As interest in combined modality approaches to cancer treatment grew, the image of the groups changed to reflect this new approach. In August of 1975 the cooperative group program was incorporated into the Division of Cancer Treatment in the interest of better coordination and centralization of therapeutic research. The CIB, thus, is a branch of the Cancer Therapy Evaluation Program (CTEP), one of four large programs of the Division of Cancer Treatment (DCT).

The groups benefitted through these successive changes, and the program has progressively expanded. Multidisciplinary expansion has occurred since 1966 as the opportunities for clinical trials of adjuvant therapy and combined modality trials became apparent. When the Division of Cancer Treatment assumed administrative responsibility for the groups in August of 1975, these efforts were intensified through supplements for multidisciplinary expansion. In FY'78 there were a total of 193 grants in 15 groups funded for $28,937,000.

The groups cover a wide range of activities and vary from large multidiscipline, multidisease study groups to single activity groups.

I. Multimodal Multidisease Groups

1. Cancer and Leukemia Group B (CALGB)
2. Eastern Cooperative Oncology Group (ECOG)
3. Southeastern Cancer Study Group (SEG)
4. Southwest Oncology Group (SWOG)

II. Multimodality Groups Devoted to a Major Oncologic Area

1. Children's Cancer Study Group
2. Gynecologic Oncology Group (GOG)

III. Single Modality Group

1. Radiation Therapy Oncology Group

IV. Single Disease Groups

1. National Surgical Adjuvant Breast Project (NSABP)
2. National Wilms' Tumor Study Group (NWTG)
3. Polycythemia Vera Group (PVG)
4. Uro-Oncology Research Group (UROG)
5. Radiotherapy Hodgkin's Disease Group (RHDG)

V. Regional Group

1. Northern California Oncology Group

VI. Special Activities Groups

1. Lymphoma Pathology Reference Center (LPRC)
2. Radiologic Physics Center (RPC)
3. Clinical Coordinating Center (CCC)
4. European Organization for Research on Treatment for Cancer (EORTC) - Operations and Statistical Office

In addition, the CIB has a functional, but not administrative, relationship with two major Veterans Administration study groups, and a third DCT contract supported group.

1. The Veterans Administration Lung Cancer Group (VALG)
2. The Veterans Administration Surgical Adjuvant Group (VASAG)
3. The Brain Tumor Study Group (BTSG)

The Type I groups are large and involve members from many disciplines: medical radiation oncology, surgery, pathology, immunology, pediatrics, psychiatry, and nursing. A statistical office provides data management and analysis for each group, with an extensive updating of results of each protocol at least every 6 months.

The Cancer and Leukemia Group B started out in the 1950's and 1960's treating only hematologic malignancies. It is now fully multimodal and has protocols for many solid tumors not previously investigated by this group, such as, oral and rectal carcinomas. A new area of interest is pyschiatry, in which patients on protocol will be studied for their reactions to their disease and its treatment.

The Eastern Cooperative Oncology Group grew out of the old Eastern Solid Tumor Study Group. It remains heavily committed to solid tumor studies, but has substantially increased its activities in combined modality studies and in hematologic malignancies.

The Southeastern Group underwent a reorganization of major proportions severa years ago. Before that, careful studies in leukemia and lymphoma were its trademark. They have now undertaken successful ventures of combined modality treatment in lung cancer and melanoma, and exhibit great potential for multidisciplinary studies in genitourinary cancer. It also has a strong nursing committee which makes contributions to biannual meetings, and are developing some of their own protocols.

The Southwest Oncology Group was a real leader in multidisciplinary organization. Largest of the groups, with an annual case accrual of over 6,000 patients, this group possesses the size and flexibility to rapidly carry out Phase III and IV studies.

The Type II groups have been multidisciplinary from their inception. Surgical and radiotherapy input has been major in the Childrens Group studies for several years. The Gynecologic Oncology Group, likewise, has developed an equal partnership of gynecologists, radiotherapists, and medical oncologists, and the majority of their protocols involve the three disciplines.

The Radiation Therapy Oncology Group, organized in 1971, conducts radiation therapy research and joint clinical investigation. They also collect long-term statistical information on radiation therapy registries. Although the RTOG is based on the application of a single therapeutic modality, they have explored the combination of radiation therapy with chemotherapy. New directions include high LET radiotherapy techniques and the use of hyperthermia and radiosensitizers.

The National Surgical Adjuvant Breast Project is one of the outstanding multimodality groups. In the past, it focused exclusively on breast cancer, but during the last year, it has expanded to doing studies on primary colorectal cancer. Some of the major contributions to our theory and practice of adjuvant chemotherapy have been accomplished by this group in the last 6 years.

The National Wilms' Tumor Study Group began as an intergroup multi-institutional program with Childrens Cancer Study Group, Acute Leukemia Group B, and the Southwest Oncology Group. An important aspect of the NWTS studies is the evaluation of the histopathology of Wilms' tumor. Another important aspect is the study of chemotherapy regimens for this tumor with the least amount of toxicity, and the analysis of epidemiology of this disease. They have made major contributions in the treatment of Wilms' tumor.

The Uro-Oncology Research Group was founded in 1974 as a consortium of Veterans Administration Hospitals. The group has formed a Surgery and Radiotherapy Evaluation Committee. The UORG is the only grant supported cooperative group of urologists wholly involved in multidisciplinary clinical trials.

The newly formed Northern California Oncology Group is the first group confined to a single state. It is composed of both academic (at Stanford) and community physicians near San Francisco.

The following special activities groups provide unusual services for their clients:

The Lymphoma Pathology Reference Center provides expert review of pathological material for the groups performing therapeutic research in malignant lymphoma.

Quality control and accurate dosimetry monitoring are vital in radiation therapy. The RPC provides the initial calibration of all radiotherapy equipment and aids the groups in the necessary continuing quality control.

The Clinical Coordinating Center is a large statistical center located at SUNYAB which provides data processing and statistical services and consultation for ECOG and RTOG, as well as contract supported organizations, the VALG and GITSG. This provides a critical mass of statistical talent. This approach has led to some noteworthy contributions to statistical science in clinical trials.

The operations and statistical office of the EORTC is also funded by the DCT. This office is the "glue" binding the multiple cooperative groups, working parties, and committees that comprise the EORTC.

In summary, the cooperative clinical groups are diverse and encompass the entire scope of cancer treatment. They comprise the entire grant-supported extramural program of the DCT, and complement the extensive intramural and contract programs of that division.

In June, 1977, the Combined Modality Branch which had responsibility for contracts, and the Clinical Investigations Branch combined to form the present CIB. Since the reorganization of the CIB, the program has disease coordinators for all diseases, as well as modality-directed staff. Essentially all protocol reviews are performed on an internal basis.

The contracts administered under CIB are divided into three categories: Phase II/III drug evaluation, disease-oriented studies by cooperative groups, and statistical support for contract-funded groups. Below is a listing of these contracts:

PHASE II/III CONTRACTS: SIGNAL TUMORS

MAYO CLINIC

LUNG
OVARIAN
CERVICAL
ENDOMETRIAL
HEAD AND NECK
PROSTATE
BLADDER
MELANOMA
SOFT TISSUE SARCOMA

M.D. ANDERSON HOSPITAL

LUNG
BREAST
MELANOMA
SOFT TISSUE SARCOMA
BONE SARCOMA

SIDNEY FARBER CANCER CENTER

LARGE BOWEL
LUNG
LYMPHOMA
HEAD AND NECK
ENDOMETRIAL
OVARIAN
BLADDER
SOFT TISSUE SARCOMA
BONE SARCOMA

MEMORIAL HOSPITAL

LARGE BOWEL
LUNG
BREAST
GASTRIC
PROSTATE
BLADDER
RENAL
OVARIAN
CERVICAL
ENDOMETRIAL
MELANOMA
PANCREAS

WAYNE STATE UNIVERSITY

LARGE BOWEL
LUNG
LYMPHOMA
HEAD AND NECK
ENDOMETRIAL
OVARIAN
BLADDER
SOFT TISSUE SARCOMA
BREAST
PROSTATE
BONE SARCOMA

STATISTICAL SUPPORT CONTRACTS: SUNYAB and Frontier Science

ADMINISTRATIVE AND OFFICE SUPPORT CONTRACT: ECTO (Georgetown University)

DISEASE-ORIENTED COOPERATIVE GROUP CONTRACTS

Gastrointestinal Tumor Study Group
Ovarian Cancer Study Group
NSABP Breast and Bowel Cancer Study
Head and Neck Cancer
Leeds, England, GI Cancer Group
Lung Cancer Study Group
WHO Melanoma Study Group

Personnel

1. Raymond B. Weiss, M.D. - Chief, CIB
2. Edwin M. Jacobs, M.D. - Associate Chief and Program Director for Grants
3. William P. McGuire, III, M.D. - Senior Investigator and Director, Contract Program
4. Jane P. Henney, M.D. - Senior Investigator and Project Officer
5. Daniel L. Kisner, M.D. - Senior Investigator and Project Officer
6. Alan Keller, M.D. - Senior Investigator and Project Officer
7. James G. Schwade, M.D. - Special Assistant for Radiation Oncology
8. J. Patterson Browder, M.D. Special Assistant for Surgical Oncology
9. Young Jack Lee, Ph.D. - Statistical Consultant
10. Ms. Elise Mackie - Program Analyst
11. Mrs. Barbara Shepherd - Secretary to Chief, CIB
12. Ms. Elaine Lewis - Secretary to Associate Chief, CIB
13. Mrs. Martha Fewell - Secretary to Drs. McGuire and Kisner
14. Mrs. Karen Best - Secretary to Drs. Keller and Henney
15. Mrs. Mary Walker - Secretary to Drs. Schwade and Browder

Dr. Weiss is responsible for the overall administration of the Branch and coordination of its activities with the Cancer Therapy Evaluation Program, the Grants Administration Branch, the Cancer Clinical Investigation Review Committee, and the National Cancer Advisory Board. He also supervises the

Project Officers on clinical contracts, and the Program Director on grants. He provides liaison between investigators of various medical disciplines within the cooperative groups and contract institutions, and encourages their participation in treatment protocols. Previously, Dr. Hugh L. Davis, Jr. acted as Chief, CIB, from May 1976 until December 1977 when he resumed his position at the University of Wisconsin. Dr. William P. McGuire, III was then made Acting Chief until Dr. Weiss' appointment in April 1978.

Dr. Jacobs serves as Associate Chief and Program Director for grants. He coordinates the program review with the Cancer Clinical Investigation Review Committee through its Executive Secretary. He is program administrator for the clinical cooperative group protocols, as well as Project Officer for the Interagency Agreements with Walter Reed Army Medical Center and the National Naval Medical Center. He is also Project Officer for Memorial Sloan-Kettering Cancer Center.

Dr. McGuire serves on the following committees: Cancer Treatment Program Staff, Clinical Trials Contract Review Committee, and the Ad Hoc Committee for Review of VA Interagency Agreement Standing Committee. He is also Project Officer and Program Director for the Lung Cancer Study Group, the Head and Neck Program, the Sidney Farber Phase II/III contract, the ECTO contract to support the Operations Office for the GITSG, LCSG, and H&N Group, and the Frontier Science contract for statistical support of GITSG.

Dr. Henney is responsible for all the breast cancer contracts, which include those with Roswell Park Memorial Institute, the Mayo Clinic, the National Cancer Institute in Milano, Italy, and the NSABP. She is also Project Officer for the Mayo Clinic Phase II/III contracts and the colon cancer contract with the NSABP.

Dr. Kisner is Project Officer for the GI cancer contracts of the GITSG, Mayo, and Leeds. He is also Project Officer for the M.D. Anderson Phase II/III contract.

Dr. Keller is the Project Officer for the Ovarian Cancer Study Group.

Dr. Schwade is the radiation oncology consultant for all CIB-administered group and contract activities. In addition, Dr. Schwade has been involved with the NCI/PAHO Collaborative Cancer Treatment Project, organizing the integration of radiation therapy-related projects into this program. Dr. Schwade's major efforts have been directed toward the organization of clinical evaluation of radiation sensitizing and protecting compounds under the Office of the Associate Director, CTEP.

Dr. Browder is the CIB consultant for all surgical oncology aspects of contract and cooperative group studies. He is the Project Officer for the Head and Neck Study contract, which involves 6 institutions and 2 cooperative groups, and was recently funded by the DCT.

Dr. Lee assists in the evaluation of statistical considerations in the review of those protocols which have complications in design.

All staff members act as disease coordinators for specific diseases, and are also representatives to specific groups.

Ms. Mackie's principal function is the undertaking of all special projects and analyses which are needed for the CIB program. She is the liaison and coordinator for all grant and contract reviews except those of the Cooperative Groups. In addition, she supervises review of protocols and maintenance of protocol files.

Mrs. Shepherd acts as administrative secretary for the Branch and is in charge of grants administration for the Cooperative Groups, working with the Program Director. She also assists the Program Analyst in preparing grant materials for review, and helps on special projects.

Ms. Lewis acts as program technical assistant and secretary to Dr. Jacobs and coordinates Cooperative Group protocol review.

Mrs. Fewell acts as assistant to the Executive Secretaries of two major contract review committees, and as such organizes meetings, monitors preparation of review materials, assists in staffing and recruitment of committee members.

Function of the CIB

Quality control is an integral part of the activities of this branch. The CIB is responsible for protocol review and for transmittal of approved protocols to the Investigational Drug Branch for filing with the Food and Drug Administration. Full reports on all studies are required, as well as an annual progress report and a report on terminal studies.

At least one CIB staff member is in attendance at each meeting of cooperative and contract groups. When appropriate, a formal program review of group and contract institutions is conducted. The CIB also acts as a coordinating center for the interaction of the groups and contractors with other NCI activities.

During the past year, the CIB has sponsored innovative multidisease-centered discussions on the following subjects:

Soft Tissue Sarcoma - 10/17/77
Sarcoma Meeting (Follow-up) - 3/17/78
Ovarian Carcinoma - 5/19/78
Pathology Working Group - 6/15-16/78
Myeloma - 7/12/78
Breast Cancer - 9/12/77
Pediatric Oncology - 4/1/78

Protocol Writing Committee Meetings were also held on Head and Neck Cancer (1/78) and Testicular Cancer (5/26/78).

Summary of Cooperative Group Efforts

In 1978, 193 grants were funded for a total of $28,937,000 as contrasted to

185 grants funded in 1977 for a total of $27,121,000.

Phase I and broad Phase II trials comprised a substantial effort in the past, but now this emphasis has changed toward Phase III and combined modality studies with curative intent (adjuvant studies).

A recent survey has indicated distinct trends: 1) the largest increase in protocols and accrual are in solid tumors, although large numbers of hematologic malignancies are still entered; 2) adjuvant studies in breast have had large accruals (e.g. 1100 patients on NSABP 04) and more than 800 patients are in followup on SWOG and ECOG colon adjuvant studies; 3) large numbers of gynecologic patients have been entered by the GOG; 4) adjuvant melanoma studies have had excellent accrual. New adjuvant trials have been activated in the GI malignancies, lung cancers, head and neck cancers, and GU cancers.

Change in direction is also indicated by specialties represented by group members. There has been a steady increase in medical oncologists and pediatric oncologists over the years, the large increase in numbers of pathologists, radiotherapists, surgeons, and other physicians in the past two years is impressive and is a direct reflection of the move toward the multidisciplinary clinical research of cancer. More than 4,000 investigators were involved in 1978.

Scientific progress is reflected in publications - 150 papers published or accepted for publication, and 114 abstracts presented and/or published at national and international meetings in 1977.

Some specific areas where noteworthy contributions have been made by the Cooperative Groups include:

1) Improved statistical methods for conducting clinical trials.
2) Definition of prognostic factors in childhood leukemia.
3) Progressively improved therapy in leukemia, with improved survival.
4) Intergroup trials in Wilms' tumor widely extending the benefit of combined modality therapy.
5) Combined modality therapy of Ewing's sarcoma, rhabdomyosarcoma, and osteosarcoma, with improved survival in these tumors.
6) Delineation of the natural history of polycythemia vera, and delineation of the therapeutic ratio of standard therapies.
7) An understanding of cell kinetics and tumor burden in myeloma.
8) Further refinement of the combined modality and chemotherapy of Hodgkin's disease.
9) Development of testing of combination chemotherapy of non-Hodgkin's lymphoma.
10) Combined modality therapy in malignant gliomas.
11) Large scale adjuvant trials in operable breast, colon, rectal cancer, and melanoma, which should define the current promise of long-term chemotherapy of micrometastases.
12) Evaluation of adriamycin and daunorubicin in AML with improved therapeutic results.
13) Large scale testing of adriamycin in solid tumors.
14) Demonstration of reduced hazard of cardiotoxicity by weekly administration of adriamycin.

15) Large scale trials of combined modality therapy of small cell lung cancer.
16) Conducted studies where exaggerated results of preliminary studies were refuted by carefully done multi-institutional trials.

Studies continue in these areas in the groups.

Overview of the Contract Program

This program started in 1972 and attempts to form small disease-oriented multimodality treatment groups. It is meant to be complimentary to the trials conducted by the large Cooperative Groups, and is not competitive with them. These programs are started whenever NCI staff feels a certain disease or treatment program needs emphasis. When a program is started, a study group is convened composed of members of Cooperative Groups, as well as non-group members. These study groups make recommendations for trials which need emphasis. If there is inability of the Cooperative Groups to conduct the needed study, then an RFP is issued for bids to perform the specific task. The contract funding for FY'78 amounted to $10,472,907 for 59 contracts. In FY'77 there were 52 contracts for a total of $9,224,685. These figures include the interagency agreements with Walter Reed, the National Naval Medical Center, and the VA Study Groups.

One of the new contracts this year is the Head and Neck Cancer Contract. The Lung Cancer Study Group has accrued about half of its needed patients, and, hopefully, will give us an answer about the value of intrapleural BCG. The GI Cancer Study Group has one of the best planned protocols to determine the value of adjuvant chemotherapy for colorectal cancer. The WHO melanoma studies have been particularly productive in answering hotly debated adjuvant surgical and chemo-immunotherapy questions in the treatment of primary disease.

Progress Summary - Contracts

Breast Cancer Contracts:

In breast cancer, two contracts (Roswell Park - Dr. Dao, and Mayo Clinic - Dr. Ahmann) are continuing to evaluate new Phase II studies and to develop more effective combination chemotherapy in advanced disease including, in one instance, an ablative procedure such as adrenalectomy. These studies represent efforts not only to identify active chemotherapeutic agents in breast cancer and combinations of active agents, but also, an increasing effort to integrate hormonal manipulations with or without chemotherapy.

1. Comparison of chemotherapy alone vs. chemotherapy plus oophorectomy.
2. Additive estrogen therapy vs. anti-estrogen therapy evaluated in both the peri-menopausal and postmenopausal group.
3. Chemotherapy vs. chemotherapy plus immunotherapy.
4. Comparison of chemotherapy alone vs. chemotherapy plus adrenalectomy.

Another major effort in breast cancer has been through contract with the Instituto Nazionale Per Lo Studio E La Cura Dei Tumori (which also includes studies in brain, lung-small cell, and testicular tumors). This contract has dealt primarily with adjuvant therapy, and its highlights have received widespread attention.

1. Establishing prolonged disease free survival in Stage II breast cancer treated with CMF.
2. Evaluating the relationship of this effect to treatment duration; i.e., 6 vs. 12 months, age, menopausal status, and number of lymph nodes.
3. The development of protocols to evaluate the Norton-Simon hypothesis by escalating chemotherapeutic agents at the times of lowest burdens.
4. Designing the initial randomized study to evaluate the role of chemotherapy in Stage III breast cancer (prior and subsequent treatment).
5. Introducing adriamycin, vincristine combination and testing its effectiveness as fixed crossover with CMF in advanced breast cancer.

During August 1977, the contract that had been awarded to the National Surgical Adjuvant Breast Project was transferred from the Breast Cancer Task Force to the Cancer Therapy Evaluation Program. The efforts of this group in exploring adjuvant chemotherapy in Stage II breast cancer are well-known. The group has become a primary force in exploring surgical questions, i.e., "best" procedure for breast cancer: radical mastectomy versus total mastectomy ± postoperative radiation, and now total versus segmental plus radiation therapy.

In the fall of 1977 the NSABP was awarded a contract to study adjuvant therapy in colorectal cancer. The group has developed two studies for patient entry during this past year. The trial in colon cancer is a prospective randomized trial of operation alone versus operation plus chemotherapy versus operation plus immunotherapy. The group has also initiated a prospective randomized trial for the adjuvant treatment of rectal carcinoma. The trial evaluates surgery alone versus surgery plus postoperative radiotherapy versus surgery plus systemic combination chemotherapy.

Head and Neck Cancer Contract:

Following the January 1978 meeting a first-draft protocol was written and circulated to all investigators. A meeting of the program staff was held on May 17, 1978 to discuss comments received from the investigators on the first draft, and a second draft was readied for circulation. Currently, the protocol embodies a two-arm structure comparing a standard treatment arm of surgery followed by postoperative radiation therapy with a study arm which adds preoperative platinum and bleomycin and postradiotherapy maintenance platinum to the standard treatment regimen. Detailed guidelines for nutritional support and pathology evaluation are also being incorporated into the protocol.

Ovarian Cancer Contracts:

The contract work in ovarian cancer has been done through four institutions with six contracts in order to study early stage ovarian cancer (Stage I & II), with a portion of the money for advanced disease work. Included is an M.D. Anderson contract for pathology review. Accrual for advanced disease studies has been acceptable, within the contract specifications, but the early stage ovarian cancer program has suffered from a poor accrual.

The contractors participated in the May 19th mini-symposium on ovarian cancer organized by the DCT. Proceedings of the symposium will be published in an upcoming issue of Cancer Treatment Reports.

Among the highlights of the ovarian contracts are:

1. Establishment of hexamethylmelamine as an active agent in ovarian cancer.
2. Introduction of cis-platinum into the treatment of ovarian cancer.
3. Defining surgical standards in early stage ovarian cancer.
4. Work on developing an ovarian carcinoma antigen.
5. Defining responses in a disease which is most difficult to evaluate.
6. Exploring the role of combination chemotherapy.

Gastrointestinal Tumor Study Group (GITSG)

This is a contract-supported clinical group with 13 institutions conducting combined modality studies in the field of gastrointestinal cancers. More than 700 patients have been accrued to GITSG protocols in the last 12 months.

Important results of GITSG studies during FY'78 include:

1. Demonstration of superiority for either 4000 rads or 6000 rads + 5-FU compared to 6000 rads alone in locally unresectable pancreatic cancer.
2. In locally unresectable gastric carcinoma, combination chemotherapy consisting of MeCCNU + 5-FU was demonstrated to produce significantly longer survival than combined modality therapy consisting of split course 5000 R + MeCCNU and 5-FU.
3. Demonstration of ineffectiveness of galactitol, ICRF-159, and βTGdR in advanced measurable previously-untreated patients with pancreatic carcinoma.

Surgical adjuvant trials in colon cancer and rectal cancer are accruing patients well, and early trends in relapse-free survival are developing but have not yet reached statistical significance. Further follow-up is required.

Mayo Clinic - Colorectal Contract

Under the direction of the principal investigator (Dr. Charles Moertel) this contract has concerned itself principally with development of new chemotherapy regimens for advanced colorectal carcinoma. During the last year, results of these trials have demonstrated the following:

1. Activity of triazinate with an 18% objective regression rate.
2. Ineffectiveness of cytosine arabinoside, metronidazole, maytansine.

A randomized Phase III trial comparing single agent ftorafur with 3 triple drug combinations is underway and will soon be analyzed. Single agent Phase II trials are presently being conducted evaluating hycanthone and chlorozotocin.

University of Leeds - Gastrointestinal Contract

This contract has contained Phase II and Phase III studies in colorectal, gastric, and pancreatic carcinomas. During FY'78 it has been demonstrated that chemotherapy consisting of MeCCNU and 5-FU significantly prolonged survival for patients with non-measurable Duke's colorectal carcinoma when compared to no treatment controls. Present Phase II efforts are in establishing the activity of chlorozotocin in previously untreated patients with advanced measurable colorectal, pancreatic, and gastric carcinomas.

The WHO Melanoma Study

They have completed an important study of the value of prophylactic regional node dissection in Stage I melanoma, which was recently published in the New England Journal of Medicine. The study did not show an advantage for this node dissection after primary tumor removal.

An on-going trial is one of adjuvant DTIC vs. BCG vs. DTIC + BCG vs. a no treatment control for Stage II melanoma. The interim report of this study shows a highly significant difference between the treated groups and the control group in disease-free survival. At this time there is no survival difference, but further follow-up is needed. 540 patients have been entered, so accrual is excellent.

Lung Cancer Study Group

A consortium of six institutions which are participating in joint protocols in resectable non-small cell lung cancer to determine the effects of various adjuvant therapies on disease-free survival. Stage I disease is a double-blind repeat of the McKneally trial using intrapleural BCG vs. no additional therapy. Stage II/III disease uses post-operative RT in squamous cell disease and compares it to no therapy and RT + levamisole. In non-squamous disease the combination of CTX/ADR/CDDP is being compared to intrapleural BCG + levamisole. The contracts have been active since September 1977 and the accrual to date is 130 - somewhat above the estimated accrual.

PHASE II/III STUDIES

Wayne State University

This contract has accrued well in the past year and the peer review committee gave its approval to continuation. Major thrust have been in colon, lung, melanoma, and sarcoma studies. Drugs currently under study are chlorozotocin, gallium nitrate, cycloleucine, VM-26, cis-platinum, ftorafur, maytansine, and rubidazone.

Sidney Farber Cancer Center

This contract has had some accrual difficulties possibly related to the newness of the contract and the institution. True Phase II studies have been in maytansine, neocarzinostatin, and cis-platinum. Some rather innovative studies on hepatic infusion with several drugs are also underway at this time, though results are not yet available.

Mayo Foundation

This contract, under the direction of Dr. John Edmonson has dealt chiefly with Phase II/III chemotherapy studies in the areas of carcinoma of the lung, head and neck, and glioma as well as malignant melanoma and osteogenic and soft tissue sarcoma. In the past year, approximately 450 patients have been entered into clinical trials.

Future plans for this contractor's work include:

1. Extend tumor sites studied to include Phase II/III trials in breast carcinoma.
2. Continue support of "supplemented" randomized study, "Postsurgical Adjuvant Therapy Osteogenic Sarcoma."

Memorial Hospital for Cancer and Allied Diseases

This contract is for Phase II/III studies to detect useful therapeutic effects of new drugs alone and in various combinations in various solid tumors. This contract has completed 30 months of a 5-year contract. Since the annual review in February 1977, 8 new protocols have been approved for inclusion under the terms of the contract, increasing the number of current studies to 18. Patient entry in this period is 533, bringing the total patient entry to 1663 in 27 protocols. Unique information has been gained from this contract in the treatment of germ cell tumors, epidermoid tumors, head and neck cancer, breast cancer, oat cell cancer, and bladder cancer.

M.D. Anderson Hospital and Tumor Institute

This contract, under the direction of Dr. Gerald Bodey has dealt chiefly with Phase II/III chemotherapy studies in the areas of carcinoma of the lung, gastrointestinal tract, head and neck, breast, and malignant melanoma. In the last year some 640 patients have been entered onto approximately 20 active protocols. The following are significant developments resulting from these trials:

1. Confirmation for synergism for actinomycin D and DTIC in advanced melanoma.
2. Establishment of activity of vindesine in advanced colorectal carcinoma.
3. Establishment of ineffectiveness of anguidine as a single agent in advanced colorectal carcinoma.
4. Establishment of activity (30% response rate) for the combination of L-asparaginase and methotrexate in advanced breast carcinoma.
5. Establishment of activity (90% PR + CR) for the combination of vincristine, iphosphamide, VP-16, and adriamycin in advanced oat cell carcinoma of the lung.
6. Demonstration that intravenous hyperalimentation during induction chemotherapy for a non-oat cell carcinoma of the lung can ameliorate hematologic and gastrointestinal toxicities of chemotherapy.

Further trials to demonstrate the effectiveness of intravenous hyperalimentation upon induction chemotherapy for non-oat cell carcinoma of the lung are continuing as well as further single agent chemotherapy trials in advanced gastrointestinal cancer.

INTERAGENCY AGREEMENTS

Walter Reed Army Medical Center (WRAMC)

This Interagency Agreement is in support of Walter Reed Army Medical Center (WRAMC) participation in the Cancer and Leukemia Group B as a full group member. WRAMC has participated actively in all the protocols of the CALGB, entering 85 patients in studies during 1977. The Principal Investigator and staff have attended all meetings and are developing multimodality functions in medical oncology, pediatrics, pathology, and radiotherapy. WRAMC staff generated six new protocols during 1977-78, and 183 new patients were entered on WRAMC protocols. The Principal Investigator has been involved in planning the Head and Neck Study Program and the Testicular Study Program. He is also a medical oncologist in the Gynecologic Oncology Group.

National Naval Medical Center

The National Naval Medical Center has participated in programs furthering the understanding of the pathophysiology of breast cancer and in improving its treatment:

1. Establishing a single multidisciplinary breast cancer clinic.
2. Establishing joint therapeutic protocols in conjunction with those at the NCI.
3. Involvement in joint laboratory studies relative to the detection and monitoring of breast carcinoma.
4. Publication and abstracts related to the contract.

This contract also contributes to interdisciplinary efforts in testicular cancer and cooperates in the Eastern Cooperative Oncology Group protocols. NNMC has participated actively in the ECOG studies, entering 22 patients.

Veterans Administration Lung Group (VALG)

In the past year 577 patients were entered on three adjuvant protocols and they have completed broad Phase II studies in the four cell types of lung cancer. No new revelations. Current studies are now divided into small cell and non-small cell, and early versus advanced disease. Accomplishments include showing the correlation between patient performance status and response to therapy.

Veterans Administration Surgical Adjuvant Group (VASAG)

VASAG has accrued 613 patients in the past year and currently has studies in colorectal, lung, esophagus, pancreas, and gastric cancer.

Major accomplishments include:

1. Identification of a small benefit of 5-FU as an adjuvant to surgery

in Duke's B and C lesions of the colon.
2. Identification of the value of preoperative radiation therapy in decreasing nodal metastases in rectal carcinoma.
3. Lack of efficacy of several single agents as adjuncts to surgery in resectable non-small cell bronchogenic carcinoma.

Current studies include evaluation of immune adjuvants in lung cancer, 5-FU, and hydroxyurea as adjuvants in colon cancer, and a comparison of the relative value of surgery and radiation therapy in esophageal carcinoma.

Future Plans

It appears that the funding of the cooperative group and contract-supported clinical trials will continue to increase as more cancer research is supported by NCI through these mechanisms. The groups and institutions are the mainstay of clinical cancer research in this country and provide good patient care and extensive professional education while providing research results.

The Board of Scientific Counselors will conduct a review of the sciences of the Cooperative Group and contract-supported clinical trials programs in April of 1979. The Group Chairmen will present the scientific achievements and future plans for the groups; the members of the Cancer Clinical Investigation Review Committee will formulate the review process of the Cooperative Groups. NCI staff will present history and review of the contract program.

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11. Bayly, T.C., Kisner, D.L., Sybert, A., Macdonald, J., Tsou, E., Shnider, B., Schein, P.: Tetracycline and quinacrine in the control of malignant pleural effusion: A randomized clinical trial. Cancer 41: 1188-1192, 1978.

12. Kisner, D.L., Davis, H.L,Jr., Muggia, F.M.: Current adjuvant trials in colorectal cancer. Frontiers in Gastrointestinal Research, 1978 (in press).

13. Davis, H.L., Kisner, D.L.: Current adjuvant trials in colorectal cancer - issues and prospects. Cancer Clinical Trials, 1978 (in press).

14. Browder, J.P., Chretien, P.B.: Immune reactivity in head and neck squamous carcinoma and relevance to the design of immunotherapy trials. Seminars in Oncology 4 (4): 431, 1977.

Staff Honors and Awards

Dr. William P. McGuire, III: Public Health Service Commendation Medal, "For unusual foresight and managerial capabilities in the strengthening of the clinical contract review system of the Division of Cancer Treatment."

Staff Presentations

Hugh L. Davis, Jr, M.D.

1. Investigations of Soviet Anti-Cancer Drugs in the United States. Fourth Annual US/USSR Oncology Program Review, Moscow, Russia, September 29, 1977.

Edwin M. Jacobs, M.D.

1. Investigational Drugs in Phase I, II, and III. Eastern Cooperative Oncology Group Meeting, Chicago, Illinois, November 1977.
2. CCIRC Review of the Southwest Oncology Group. Southwest Oncology Group Meeting, Phoenix, Arizona, September, 1977.
3. CCIRC Review of the Eastern Cooperative Oncology Group. Eastern Cooperative Oncology Group Meeting, Madison, Wisconsin, June 1978.

4. NCI Reorganization; Funding; Protocol Review. Northern California Oncology Meeting, Palo Alto, California, May 1978.

William P. McGuire, III, M.D.

1. U.S. Trials in Lung Cancer. International Association for the Study of Lung Cancer, Hilton Head, N.C., May 12, 1978.

Daniel L. Kisner, M.D.

1. Malignant Cachexia: Insulin Resistance and Insulin Receptors. American Association for Cancer Research, Washington, D.C., April 1978.

Alan M. Keller, M.D.

1. A Historical Overview of Cinical Trials in Non-Hodgkin's Lymphoma. (with Delia Chiuten, M.D.). Intergroup Non-Hodgkin's Lymphoma Conference, Bethesda, MD, February 1978.
2. Gastric Lymphoma - A Review. Veterans Administration Hospital, Washington, D.C., March 1978.
3. Bladder Cancer - Diagnosis, Staging, and Treatment. Veterans Administration Hospital, Washington, D.C., April 1978.

James G. Schwade, M.D.

1. Treatment of Spinal Cord Neoplasms. American Society of Radiologists, Denver, Colorado, October 1977.
2. Radiation Sensitizers. Brooke Army Medical Center, San Antonio, Texas, May 1978.

Jane E, Henney, M.D.

1. Planning Future Protocols for Patients with Primary Cancer - Overview of Ongoing Protocols. National Surgical Adjuvant Breast Project Meeting, Miami, Florida, October 1977.
2. Platinum Compounds - Medical Uses. EUCHEM Conference, Satellite Symposium of the Seventh International Congress of Pharmacology, Toulouse, France, July 1978.

Combined Modality Contract Review Committee
National Institutes of Health
National Cancer Institute

Function:

The Combined Modality Committee shall advise the Director, National Cancer Institute and the Director, Division of Cancer Treatment concerning the scientific merit of proposals for contracts submitted to the Division of Cancer Treatment. As experts in their fields, the committee members shall survey the status of cancer treatment and recommend areas of research that should be supported by the Division of Cancer Treatment.

Dates and Places of Meetings:

1. November 14-15, 1977 - Building 31, NIH, Bethesda, Maryland
2. February 21, 1978 - Building 31, NIH, Bethesda, Maryland

Summary of Activities:

1. November 14, 1977:

This meeting was held for the purpose of reviewing responses to RFP N01-CM-87154 adjuvant chemotherapy trial in head and neck squamous carcinoma. Twenty two responses were reviewed for technical merit by the committee and a group of ad hoc advisors. Nine proposals were found to be technically acceptable by the committee with the specifics of the contract to be negotiated by NCI staff. Committee recommended deleting a protocol for unresectable lesions from the workscope of the project.

November 15, 1977:

The committee was reconvened on this day to review renewal proposals from the Gastrointestinal Tumor Study Group participants in clinical trials for patients with gastric and pancreatic carcinomas. Accrual had lagged somewhat behind expectations in several institutions and recommendations were made for appropriate reductions in budgets.

A. Gastric Contract Renewal Proposals

1. Mt. Sinai School of Medicine - N01-CM-53841
   Renewal approved.
2. Yale University - N01-CM-53843
   Renewal approved.
3. N.Y.S.D.H./Health Research - N01-CM-43782
   Renewal approved.
4. Sidney Farber Cancer Institute - N01-CM-43781
   Renewal approved.
5. University of Southern California - N01-CM-53842
   Renewal recommended for disapproval because of low accrual.

B. Pancreatic Contract Renewal Proposals

1. Yale University - N01-CM-43797
   Renewal approved.
2. Mayo Foundation - N01-CM-43796
   Renewal approved.
3. University of Miami - N01-CM-43800
   Renewal approved.
4. N.Y.S.D.H./Health Research - N01-CM-43794
   Renewal approved.
5. University of California, Los Angeles - N01-CM-43798
   Renewal disapproved because of inadequate accrual.
6. Sidney Farber Cancer Institute - N01-CM-43799
   Renewal disapproved because of inadequate accrual.

II. February 21, 1978

The purpose of this meeting was to evaluate a renewal proposal for breast and gastrointestinal studies done at the National Cancer Institute of Milan, renewal proposal for a coordinating center for the WHO melanoma study, responses to RFP NCI-CM-87194 support services for clinical trials, responses to RFP NCI-CM-87193 statistical support for a cooperate group.

1. NCI - Milan Clinical Trials for Breast and Gastrointestinal Carcinoma - N01-CM-33714
   Renewal approved.
2. Coordinating center for clinical study of melanoma (WHO) N01-CM-43726
   Renewal approved.
3. Support services for clinical trials RFP NCI CM-87194 Two responses were reviewed by the committee with one being found technically acceptable.
4. Statistical support for a cooperative group engaged in intensive studies and investigation on patients with gastrointestinal carcinoma NCI CM-87193

The committee reviewed four proposals for technical merit finding two technically acceptable and two technically unacceptable.

APPENDIX II - ANNUAL REPORT

Clinical Trials Contract Review Committee
National Institutes of Health
National Cancer Institute

Function:

The Clinical Trials Committee shall advise the Director, National Cancer Institute, and the Director, Division of Cancer Treatment concerning the scientific merit of proposals for contracts submitted in the areas of clinical trials to the Division of Cancer Treatment. As experts in their field, the committee members shall survey the status of cancer treatment in recommended areas of research to be supported by the Division of Cancer Treatment.

Dates and Places of Meetings:

1. December 15, 1977, Building 31, Conference Room 8, NIH, Bethesda, MD
2. March 30-31, 1978, Building 31, Conference Room 8, NIH, Bethesda, MD

Summary of Activities:

1. December 15, 1977:

Two unsolicited proposals were reviewed as the initial order of business. The first was from Dr. Budinger, a proposal for hyperthermia experimentations in large animal (canine) model and the second from Dr. Pettigrew for hyperthermia clinical trials. Both of the unsolicited proposals were recommended for approval with provisions of site visits.

Two renewal proposals were then reviewed:

a. N01-CM-53832, "Quantitative Evaluation of Protective Environment" M. D. Anderson Hospital. Renewal approved only for the studies conducted in breast cancer.

b. N01-CM-71343, "Uganda Cancer Institute" - Renewal funding approved.

The committee then considered the accrual of patients on two of the Phase II - III contracts. This data had been requested by the committee at its March 1977 meeting. The accrual data from both the Mayo Foundation and Wayne State University were found to be acceptable by the committee.

The final item of business was the interim report contract N01-CM-33710 "Clinical Trials in Advanced Ovarian Cancer" - M. D. Anderson Hospital. It was recommended that the contract be continued, but a re-assessment made at the time of the annual report in March 1978.

2. March 30, 1978:

The first item of business was an update regarding the current status of unsolicited proposals considered at the December 1977 meeting. It was the recommendation of the site visitor of Dr. Budinger's laboratory that this proposal be disapproved. The committee concurred with this recommendation. Protocols had been recently received from Dr. Pettigrew and plans were made to circulate these protocols for review by the committee members. Protocols for clinical trials conducted within the protected environment contract N01-CM-53832 etc., have recently been received at the NCI. These protocols were circulated to both NCI staff and representatives from the Clinical Trials Contract Review Committee to the Division of Cancer Research Resources and Centers.

Annual Reports and Renewal Proposals were then considered:

A. "Adjuvant Studies in Patients with Colorectal Cancer"
University of Pittsburgh - N01-CM-7177 - Renewal Approved.

B. "Clinical Trials in Primary Breast Cancer"
University of Pittsburgh - N01-CB-3876 - Renewal Approved.

C. "Therapy of Advanced Ovarian Cancer"
M. D. Anderson - N01-CM-33710 - Renewal Approved.

D. Brain Tumor Study Group
Renewal Approved for all institutions.

E. Ovarian Cancer Study Group
Renewal Disapproved for all groups.

F. Phase II-III Working Group
Sidney Farber Cancer Institute - N01-CM-67037 - Renewal Approved.
Memorial Sloan-Kettering Hospital - N01-CM-57043 - Renewal Approved.
Mayo Foundation - N01-CM-57044 - Renewal Approved.
Wayne State University - N01-CM-67105 - Renewal Approved.

3. March 31, 1978:

The first item of business discussed was that of the work being conducted by the Lung Cancer Study Group. Each protocol was discussed in length. All institutions participating in this group were renewed for the following year.

Phase II-III Working Group (continued)

M. D. Anderson Hospital and Tumor Institute - N01-CM-57042 - Renewal Approved

The committee then discussed tentative dates for next years meetings. December 8-9, 1978 and March 29-30, 1979 were found to be suitable.

INVESTIGATIONAL DRUG BRANCH

The Investigational Drug Branch has the mission of sponsoring new investigational drugs for clinical trials and of evaluating them for antitumor efficacy. It does this by pursuing several objectives: (a) obtaining Investigational New Drug exemption (IND) authorization from the Food and Drug Administration (FDA), (b) managing the Phase I Working Group, (c) monitoring the clinical trials conducted by these investigators, (d) meeting FDA regulatory affairs responsibilities for all active IND's and, (e) regulating the distribution of investigational new drugs.

The permanent professional staff of the Branch include one physician, one pharmacologist, one biochemist, and one pharmacist. There are two staff associate physicians, two visiting fellow physicians, and one visiting scientist who spend one of a two year appointment with the Branch.

During the past year the name of the previously existing Drug Liaison and Distribution Section was changed to the Drug Regulatory Affairs Section and a new section, the Drug Evaluation and Reporting Section was created.

One of the most significant achievements of the past year was the creation of a new system of drug classification and the development of clear policies for DCT sponsorship of protocols for investigational drugs. In a series of meetings with the Bureau of Drugs, FDA the details of a document "Operational Plan and Procedures for Clinical Trials" were negotiated and submitted to FDA as a Master Plan. Briefly, drugs are classified as either Group A, B, or C. Group A drugs are those in Phase I clinical trials or in their initial Phase II trials. Protocols are accepted from members of the Phase I Working Group, DCT Contractors, or Cooperative Groups. Group B drugs are those which have been evaluated for efficacy in several Phase II trials in pertinent tumors and which have been shown in these studies to be tolerated by patients. Protocols are accepted from DCT Contractors, Cooperative Groups, and through the New Drug Studies mechanism. This last source was implemented in the fall of 1977 and is described below in more detail. Group C drugs have reproducible efficacy in specific types of tumors. These drugs are provided to registered Physicians for treatment of patients with specified diseases in accord with our "Guidelines" protocols. Safety of use is the research objective of this mechanism.

DRUG REGULATORY AFFAIRS SECTION

Food and Drug Administration Activities

Investigational New Drug (IND) Notices: There were seven new IND notices prepared and submitted to the Bureau of Drugs, FDA in 1977. Clearance of these IND notices by FDA allowed clinical studies to begin. The drugs are:

Levamisole (NSC-177023)
Misonidazole (Ro-07-0582, NSC-261037)
Bruceantin (NSC-165563)
AMSA (NSC-249992)
Indicine-N-Oxide (NSC-132319)
Pyrazolo-Imidazole (IMPY, NSC-51143)
PALA (NSC-224131)

During 1978, one IND has been submitted and approved by FDA. This is for the drug L-Alanosine (NSC-153353). In addition, six IND notices are currently in preparation. These are:

Cytotoxic Agents

PCNU
Pentamethylmelamine
DON
5-Methyltetrahydrohomofolate
Soluble ICRF-159

Others

Heroin

Drug Distribution Activities: There are currently 18 drugs classified as Group A, 62 drugs as Group B, and 7 drugs as Group C. Requests for these drugs are processed through a verification system which insures that investigational anticancer drugs are distributed efficiently and in conformance with Food and Drug Administration requirements.

Other Activities

In calendar 1977, 269 new research protocols were approved, recorded, and authorized for receipt of clinical drugs. Based upon protocol specifications, forecasts of clinical drug needs were routinely made as an aid to the overall planning process. The Section is also responsible for the registration of investigators permitting the use of Group C drugs.

Two support service contracts are under the Section's management and supervision. The first provides data processing of drug verification and distribution information; the second assists in the conduct of Regulatory Affairs functions.

DRUG EVALUATION AND REPORTING SECTION

Phase I studies: Seven investigational drugs sponsored by the DCT were newly introduced into Phase I Clinical Trials:

Alanosine	NSC-153353
AMSA	NSC-249992
Bruceantin	NSC-165563
Pyrazolo Imidazole	NSC-51143
Indicine-N-Oxide	NSC-132319
Misonidazole	NSC-261037
PALA	NSC-224131

In addition Phase I studies were continued last year on the following drugs:

Cycloleucine	NSC-1026
Chlorozotocin	NSC-178248
Dichloro-Lawsone	NSC-126771
Dichloromethotrexate	NSC-29630
Deazauridine	NSC-126849
Gallium Nitrate	NSC-15200
Maytansine	NSC-153858
Neocarzinostatin	NSC-157365
Rubidazone	NSC-164011

The Phase I studies are conducted by members of the Phase I Working Group, which consists of clinical investigators from five Branches of the NCI Intramural Program, seven institutions with Phase I contracts, and several institutions conducting Phase I studies without financial support. Whenever methodology allows pharmacokinetic studies in man have been conducted. Detailed information on the status of these studies is available to interested investigators.

Single agent Phase II Studies on Group A drugs are being conducted by DCT Contractors and Cooperative Groups. The following studies are underway:

Hycanthone (NSC-142982): Breast cancer, Colorectal cancer, and lymphoma, Anguidine (NSC-141537): Cancer of the: Breast, Colon & rectum, Urinary Bladder, Gall Bladder, Stomach, Kidney, Pancreas, Prostate, and Testicle, ALL, Brain tumors, and Sarcoma,

Chlorotozotocin (NSC-178248): Cancer of the Breast, Colon & rectum, Lung, Stomach, and Pancreas, Melanoma, Sarcoma, and Myeloma,

Maytansine (NSC-153858): Cancer of the: Breast, Colon & rectum, Lung, Head & neck, Ovary, Pancreas, and Testicle, Lymphoma, Melanoma, and Sarcoma,

Neocarzinostatin (NSC-157365): Cancer of the: Urinary bladder, Liver, and Testicle, ALL, AML, and Melanoma,

Dichloromethotrexate (NSC-29630): Cancer of the: Colon & rectum, Lung, Gall bladder, and Liver,

Rubidazone (NSC-164011): Cancer of the Breast and Lung, AML, Lymphoma, and Osteosarcoma.

The status of these studies are reviewed semiannually at the New Drug Liaison Meetings. In addition the IDB professional staff provides a liaison function at cooperative group meetings, contractor meetings, and disease oriented discussion and coordination meetings organized by the CTEP.

NEW DRUG STUDIES MECHANISM

In August, 1977 a mechanism for studying new drugs, other than through NCI contract or grant support, was instituted. The FDA approved the program. The new mechanism allows Comprehensive Cancer Centers and Clinical Cancer Centers supported by the Cancer Center Support (CORE) Grant to approve and sponsor protocol studies on selected drugs. These drugs include: Baker's Antifol, β-TGdR, Camptothecin, Cyclocytidine, Dianhydrogalactitol, Dibromodulcitol, Dibromomannitol, Diglycoaldehyde, Elliott's B Solution, ICRF-159, Melphalan (i.v.), TMCA, VM-26, VP-16-213, Yoshi 864, Azacytidine, Cis-Platinum, Daunomycin, Hexamethylmelamine, Erwinia L-Asparaginase, MeCCNU, and Streptozotocin.

There are 49 organizations eligible to participate in this program. Thirty-seven have registered with the IDB indicating their willingness to participate in the mechanism.

Thirteen of the 37 institutions now have a total of 47 active protocols. A breakdown of these includes:

Institution	Number of Protocols
University of Arizona	2
Colorado Regional	2
Yale Comprehensive	1
Johns Hopkins	3
Mayo Clinic	9
Sidney Farber	2
Ohio State	8
Fox Chase	2
University of Texas	2
St. Jude Children's	1
University of Chicago	2
University of Wisconsin	7
Florida Comprehensive	1

Drugs under study include: Baker's Antifol, VP-16-213, TMCA, ICRF-159, Dianhydrogalactitol, Dibromodulcitol, Methotrexate, Azacytidine, MeCCNU, Hexamethylmelamine, Cis-Platinum, and Levamisole.

Most Cancer Centers have voluntarily accepted regional responsibility for drug studies. The areas of voluntary responsbility are:

GEOGRAPHICAL REGIONS VOLUNTARY ESTABLISHED BY CANCER CENTERS

NCI Number	Cancer Center	Designated Area
1	University of Alabama Comprehensive Cancer Center	State of Alabama and City of Pensacola, Florida
3	UCLA Comprehensive Cancer Center	Southern California and Las Vegas, Nevada area
4	Northern California Cancer Program	
5	Colorado Regional Cancer Center, Inc.	States of Colorado, Wyoming, portions of Eastern Montana, Western South Dakota, Western Nebraska, and Western Kansas

NCI Number	Cancer Center	Designated Area
6	Yale University Comprehensive Cancer Center	State of Connecticut, Northeastern part of Westchester County, New York and the Springfield area of Massachusetts
7	Vincent T. Lombardi Research Center, Washington, D. C.	
8	Howard University College of Medicine	Washington, D. C. and metropolitan area
9	Comprehensive Cancer Center for the State of Florida	South Florida
10	Emory University Cancer Center	State of Georgia, several investigators from Southern and Western South Carolina
11	University of Hawaii	State of Hawaii
14	University of Chicago Cancer Research Center	
17	Johns Hopkins Oncology Center	Center physicians and community physicians who hold Center appointment
18	Sidney Farber Comprehensive Cancer Center	States of Massachusetts and Maine
19	Boston University Cancer Research Center	Staff of Boston University Medical Center composed of University Hospital, Goldman School of Graduate Dentistry and School of Medicine
20	Mayo Comprehensive Cancer Center	Members of Staff; North Central Cancer Treatment Group
21	Missouri Cancer Programs	
24	Sloan Kettering Institute for Cancer Research, New York	

NCI Number	Cancer Center	Designated Area
26	Roswell Park Memorial Institute	State of New York with exception of New York City Northern & Western Counties of Pennsylvania and Northeastern & Eastern Counties of Ohio
27	Cancer Research Center, Bronx, New York	The Bronx, Westchester County & affiliated hospitals in Brooklyn and New Jersey
31	University of Rochester Cancer Center, Rochester, New York	Nine counties of Genesee-Finger Lakes Health Systems Agency will consider investigators in Eastern part of New York
32	Duke University Comprehensive Cancer Durham, North Carolina	Members of Duke Comprehensive Cancer Center, members of faculty of Duke Medical Center members of Eastern Oncology Network
34	Oncology Research Center for Winston-Salem, North Carolina	
35	Ohio State University Comprehensive Cancer Center	State of Ohio with the exception of Northern Ohio counties
36	The Cancer Center, Inc., Cleveland, Ohio	Counties of Ashtabula, Columbiana, Geauga, Cuyahoga, Lake, Mahoning, Medina, Portage, Stark, Summit, and Trumbull, Ohio
37	Oklahoma Cancer Center	State of Oklahoma
38	Fox Chase Cancer Center	Programs of Fox Chase
39	University of Pennsylvania Cancer Center	Cities of Philadelphia, West Chester, Hazelton and Williamsport, Penna. and Willingboro, New Jersey
41	Roger Williams General Hospital, Providence, Rhode Island	

NCI Number	Cancer Center	Designated Area
42	Memphis Regional Cancer Center, Memphis, Tennessee	Memphis, Shelby County, Tennessee; Crittenden County, Arkansas; and DeSota County, Mississippi
43	University of Texas System Cancer Center, Houston, Texas	
44	University of Texas Health Science Center, Dallas, Texas	North Texas area and immediate 8 county Dallas-Fort Worth Standard metropolitan area
47	Fred Hutchinson Cancer Research, Seattle Washington	States of Washington, Alaska, Montana, Idaho, and Oregon
48	University of Wisconsin Clinical Cancer Center, Madison, Wisconsin	Most of State of Wisconsin and adjoining aspects of Michigan, Iowa, and Illinois
49	Medical College of Wisconsin, Milwaukee, Wisconsin	Entire midwest with reference to childhood cancer only
50	St. Jude Children's Research Hospital, Memphis, Tennessee	
51	Stanford University Medical Center Stanford, California	
52	University of California, San Diego, California	San Diego and Imperial Counties

Recently, the need to allow institutions other than Cancer Centers to participate in this mechanism has been addressed. A new policy has been formulated which would allow other institutions to participate in this mechanism. Briefly approved unfunded applicants to the Cancer Center Support (CORE) Grant (P-30) are recognized as potential protocol approving authorities, other institutions meeting exacting requirements (enumerated in the Guidelines for Application for Participation in the New Drug Studies mechanism) are also eligible for participation. It is anticipated that this new direction will greatly increase the utilization of this mechanism.

CONTRACT ACTIVITIES

These contracts are designed to conduct Phase I Clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, NCI or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. All of these contracts end on September 30, 1978 and will be replaced on the basis of competition awards.

SIDNEY FARBER CANCER INSTITUTE (NO1-CM5-3839). Phase I studies have been conducted on Maytansine (NSC-153858), Neocarzinostatin (NSC-157365), Bruceantin (NSC-165563), PALA (NSC-224131), and 5-fluorouracil with thymidine during the past year.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NO1-CM4-3801). Phase I studies have been conducted on Neocarzinostatin (NSC-157365), Deazauridine (NSC-126849), Hycanthone (NSC-142982), Dichloroallyl lawsone (NSC-126771), m-AMSA (NSC-249992), Bruceantin (NSC-165563), Imidazole pyrazolo (IMPY, NSC-51143), and PALA (NSC-224131), during the past year.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM5-3837). Phase I studies have been conducted on Neocarzinostatin (NSC-157365), Dichloromethotrexate (NSC-29630), Maytansine (NSC-153858), m-AMSA (NSC-249992), PALA (NSC-224131), Indicine-N-Oxide (NSC-132319), and two drug combinations pyrazofurin plus azacytidine and 5-fluorouracil and thymidine during the past year.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM4-3804). Phase I studies have been conducted on Chlorozotocin (NSC-178248), Gallium Nitrate (NSC-15200), Rubidazone (NSC-164011), m-AMSA (NSC-249992), PALA (NSC-224131), and the combination of 5-fluorouracil and thymidine during the past year.

MAYO FOUNDATION (NO1-CM5-3838). Phase I studies have been conducted on Hycanthone (NSC-142982), Chlorozotocin (NSC-178248), Indicine-N-Oxide (NSC-132319), and Alanosine (NSC-153353) during the past year.

KANSAS, UNIVERSITY OF (NO1-CM4-3802). Phase I studies have been conducted on Anguidine (NSC-141537), Chlorozotocin (NSC-178248), and Imidazole Pyrazole (IMPY, NSC-51143) during the past year. Studies on Bruceantin (NSC-165563), and ICRF-187 (NSC-169780) are being planned.

CHILDREN'S HOSPITAL OF LOS ANGELES (NO1-CM4-3803). Phase I studies have been conducted on β-deoxythioguanosine (NSC-71851), Neocarzinostatin (NSC-157365), and Imidazole pyrazolo (IMPY, NSC-51143) during the past year.

The following contracts and CREG's investigate the biochemical and molecular biological action of investigational new drugs being developed by the DCT Program.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-3784). The work scope of this contract has been subdivided into five project objectives: (1) To determine if new antitumor drugs have biochemical mechanisms of action similar to those of clinically established antitumor drugs. (2) To identify or provide clear leads to the biologically significant biochemical properties of new antitumor drugs. (3) To identify and study the molecular mechanism of action of new antitumor drugs. (4) To select from among active structural analogs of clinically evaluated antitumor drugs, those which may have more desirable biochemical properties. (5) To develop and evaluate biochemical rationales for the combined use of clinically established antitumor drugs. This contractor has published several papers resulting from this contract, and is conducting outstanding investigations. Information gathered from this effort has been very useful in preparing Biochemical Data Sheets. This is one of the most productive groups in the biochemical research area. This contract was terminated on March 31, 1978. However, the Principal Investigator has successfully competed for CREG support for similar studies.

M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NO1-CM4-3786). The objective of this project was to provide a detailed study of selected drugs in development for clinical trials on polynucleotide metabolism. This contract was terminated on March 31, 1978.

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-CM4-3787). The major emphasis of this project was to provide a detailed evaluation of new antitumor drugs which affect protein synthesis. Studies with Bruceantin and Anguidine have been completed and published. In depth studies are being conducted with VP-16 (NSC-141540). This contract was terminated on March 31, 1978. However, the Principal Investigator has successfully competed for CREG support for similar studies.

BRIGHAM YOUNG UNIVERSITY (NO1-CM4-3790). The objective of this project was to provide detailed information on the effects of selected antitumor drugs on folate metabolism. This contract was terminated on March 31, 1978.

CLINICA PUERTA DE HIERRO (NO1-CM5-3792). The objective of this contract was to study the details of the effects of anticancer agents on electron transport processes. This contract was terminated on March 31, 1978.

UNIVERSITY OF MINNESOTA (NO1-CM4-3788). The major emphasis of this project is directed toward determining the cell uptake transport processes of selected antitumor drugs. In addition, the effects of membrane active, natural substances on the uptake and effectiveness of antitumor drugs was being evaluated. This contract was terminated on March 31, 1978.

MICHIGAN CANCER FOUNDATION (NO1-CM5-3789). The objective of this project was to provide detailed information on the effects of antitumor agents on membrane properties and structure. This contract was terminated on March 31, 1978. However, the Principal Investigator has successfully competed for CREG support for similar studies.

UNIVERSITY OF PENNSYLVANIA (NO1-CM5-3830). The general objective of this project was to evaluate the effects of selected antitumor agents on the bioenergetic performance status of intact organs. The specific research has been directed toward determining the mechanism of adriamycin cytotoxicity. More recently Dr. Chance has observed that adriamycin stimulates the formation of superoxide anion radical in mitochondria in vitro, raising the possibility of secondary damage due to this species. This contract was terminated on March 31, 1978.

HARVARD UNIVERSITY (NO1-CM5-3825). The objective of this project is to evaluate the role of carbohydrate metabolism in the development potential of chemotherapeutic agents.

It was found that the nucleoside activation of glucose and mannose were sensitively dependent on the prior carbohydrate nutrient history of the cell line (e.g., glucose, mannose, or sugar deprivation). At concentrations having no effect on cell growth or RNA or protein synthesis, 6-thioguanine and 6-thioguanosine significantly inhibited the conversion of glucose and to UDP-glucuronic acid.

In vivo, mannose, glucose, and galactose all failed to increase the survival of P388 leukemia bearing mice. However, combinations of deoxyglucose and 6-thioguanine did increase the mean survival time and produced long term survivors. This effect appears to be a synergistic one. These studies were expanded to include deoxygalactose. This was contract was extended without funds until October 31, 1978 and will then terminate.

NEW YORK STATE DEPARTMENT OF HEALTH (NO1-CM4-3785). The "Profile of Drug Interaction" system under development is designed to (a) determine the similarity of new anticancer agents to agents of known mechanism of action, (b) to determine whether or not all the agents in a series of structural analogs are also biochemical analogs. This contract was terminated on September 30, 1977. A summary of the work indicates that:

1. Compounds which cause gross disruption of DNA function by binding to, alkylating or incorporating into DNA produce

an additive interaction when evaluated in combination with most other drugs.

2. Most of the drugs in the panel displayed unique profiles.

3. A much larger panel of drugs than originally proposed (34) is required to adequately characterize each agent.

4. The system can distinguish between a purine or pyrimidine analog which inhibits growth through incorporation into DNA or by action at some earlier precursor step.

BIOTECH RESEARCH LABORATORIES, INC. (NO1-CM6-7052). The objective of the project whose progress is reported here is to set up and apply an in vitro primary screening system in which compounds are tested for their possible value as chemotherapeutic agents against human neoplasms by testing their effect on the functioning of oncogenic viruses. The screening system used in the project screens for compounds which selectively inhibit the following: (1) transformation of cells by a DNA or RNA oncogenic virus, (2) growth of transformed cells relative to the untransformed counterpart, (3) expression of an antigen associated with infection and transformation by a DNA or RNA virus, and (4) activity of purified RNA-dependent DNA polymerase.

One hundred and fifty drugs have been subjected to this screen. Twelve compounds have activity against the RNA virus system while four others have activity against the DNA virus. Further analysis of this data is being undertaken. This contract was terminated June 30, 1978.

WASHINGTON UNIVERSITY (NO1-CM4-3731). This project has as its objective the study of the effects of combinations of antitumor agents on normal and malignant cell populations in the mouse. Quantitative cellular assays are used and particular attention has been placed on the role of drug sequence and drug interval The agents chosen for study (based on activity in human breast cancer) were adriamycin, cytoxan, 5-FU, methotrexate L-PAM, vincristine, and x-ray. This contract was terminated on March 31, 1978.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (NO1-CM1-0056). This is an interagency agreement and is in the terminal year (December 31, 1978).

The major goal of this program is the development of a cultured cell model system which can predict inexpensively, easily, and accurately the type of kinetic response occurring in vivo when susceptible tumor cell populations are exposed to cancer chemotherapeutic agents.

Models for both cycling and noncycling cells are provided by employing exponentially growing cultures of line CHO cells to represent cycling tumor cells, while initially noncycling tumor cells are represented by isoleucine-deprived G_1-arrested CHO cells stimulated to return to cycle by resuspension in completed medium at the time of drug removal.

Recent studies have been aimed at examining the complementary types of data obtainable from FMF and chromosome damage studies of cells treated with anticancer drugs.

Determination of the non-cycle-traversing subpopulations (via FMF analysis) and the cycling subpopulations sustaining maximal chromosome damage (via karyotype analysis) allows identification of the irreversibly injured cells and, by difference, the potential survivors which are of clinical importance.

For example in studies with m-AMSA chloride FMF analysis revealed that (a) noncycling G_1-arrested cells were highly resistant to the effects of m-AMSA on both survival and cell-cycle progression capacity, and (b) cycling cells treated with m-AMSA were characterized by a subpopulation of resistance cell initially in G_1 which were much less affected in their capacity for cycle progression than cells in S and early to mid-G_2 phase during the period of exposure to m-AMSA.

The following new grants (CREG) were awarded on April 1, 1978 for the biochemical and molecular biological evaluation of invetigational new drugs being developed by the DCT Program.

UNIVERSITY OF TEXAS SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (1RO1 CA-23272-01). Benjamin Drewinko - Principal Investigator. This project will analyze the lethal and kinetic effects of new antitumor agents on various established human cell lines as a function of drug concentration, exposure time, and position of the cells in the cycle at the time of drug administration (cell cycle stage-dependent function). Cell kill will be assessed by the reduction in colony-forming capacity. Effects on cycle progression will be defined by sequential DNA histograms obtained by pulse cytophotometry. Combination chemotherapy using drug pairs on synchronized cells will be conducted to define the mechanism of cell killing potentiation. Paired treatment will be conducted in simultaneous or staggered fashion. Other experiments will analyze the effects of sequential treatment of asynchronous cells with two antitumor drugs. The first drug will be used as a synchronizing agent while the second drug will be used as a cytotoxic agent. These investigations on drug effects at the cellular level should contribute a rationale guideline for developing superior clinical therapeutic protocols.

UNIVERSITY OF TEXAS SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (1RO1 CA-23270-01). Raymond E. Meyn - Principal Investigator. The overall objective of this research is to characterize the cell cycle phase-dependent sensitivity of new anticancer drugs both *in vitro* and *in vivo*. These studies will employ a new method for selection of synchronized mammalian cells, centrifugal elutriation separation, which has several distinct advantages over conventional synchrony techniques. The ease and speed of the technique and the degree of synchrony obtained makes testing the cell cycle stage sensitivity simple and independent of pertubations caused by induced synchrony techniques. The cell cycle stage sensitivity will be assayed for several different cell lines in culture, including a fibrosarcoma line which also grows in mice as a solid tumor. The same cell line will be assayed for its cell cycle stage sensitivity to drugs *in vivo* and the results will be compared to the *in vitro* studies.

SLOAN KETTERING INSTITUTE FOR CANCER RESEARCH (1RO1 CA-23296-01). Z. Darzynkiewicz - Principal Investigator. The primary objective of this study is to characterize the cell cycle effect of new anticancer drugs used singly or in combinations. Approximately 20 new drugs or drug combinations, to be chosen in consultation with the Division of Cancer Treatment, NCI, will be studied each year by both flow cytometry and autoradiography. A second objective is to provide information on the mechanism of action of the drugs, based on their effect on cell cycle kinetics and possible effects on nuclear chromatin conformation studied *in situ* by new developed flow cytometry techniques.

The effect of each drug (or drug combination) will be studied over an appropriate range of concentrations and time intervals on 4 different cell types: SK-L7 human leukemia cells; Friend murine leukemia cells; normal human peripheral blood lympocytes; and Ehrlich ascites HD33 tumor. Thus the drug effects can be compared on leukemic cells and normal lymphocytes, and on hemopoietic vs. non-hemopoietic cells.

JOSEPH STOKES, JR., RESEARCH INSTITUTE, CHILDREN'S HOSPITAL OF PHILADELPHIA (1RO1 CA-23147-01). William A. Creasey - Principal Investigator. The aim of this project is to identify mechanisms of action of new drugs. Primary mouse tumor systems used are sarcoma 180, leukemia L1210, and a neuroblastoma model. Cultured human cell lines of neuroglastomas and brain tumors are also available for some experiments. Incorporation of radiolabeled precursors is followed *in vivo* and *in vitro*: thymidine and uridine into nucleotides and nucleic acids; phenylalanine into the proteins; acetate into lipids; and these precursors and amino sugars into membranes. The measurement of the integrity of nucleic acids are made by neutral and alkaline sucrose density gradient ultracentrifugation. Cell free systems of ribosomes and synthetic messenger are used to study protein synthesis *in vitro*. Intracellular uptake, distribution, and metabolism of drugs are studied by high-pressure liquid chromatography, fluorometry, and spectrophotometry. Changes in the function and integrity of the cell membrane are assayed by transport of metabolities and binding of and agglutination by concanavalin A. Microtubule protein is measured by colchicine binding assay using DEAE, and competition by other drugs determined. Transport of drugs will be studied in cell suspensions, using a spin through isotonic sucrose to separate the cells from drug-containing medium.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03579-05 LMCB

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Effect of Chemotherapeutic Agents on L1210 Cell Cycle Progression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI's:	Vincent H. Bono, Jr.	Chief	IDB, NCI
	Robert L. Dion	Chemist	LMCB, NCI

COOPERATING UNITS (if any)
The Upjohn Company, Kalamazoo, Michigan, Cancer Research Contract NO1-CM-43743

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 1/4	1 1/4	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project is designed to investigate the effects of cancer chemotherapeutic agents on cell cycle progression based on their cytotoxicity in an in vitro L1210 murine leukemia system. DNA content distribution (DCD) curves are generated with the use of a flow microfluorometer and drug treated curves are compared with steady state DCD curves after analysis by computer. An attempt to classify these agents as to their mode of action (anti-metabolite, etc.) by computer analysis is in progress.

PHS-6040

Project Description:

This project is concerned with development of techniques for the analysis of drug effects on the progress of cells through the proliferative cell cycle and their application to (a) the characterization of therapeutically active drugs and (b) the identification and possible action of agents with potential therapeutic activity. The major methods employed are (a) mammalian cell culture (L1210 murine leukemia in suspension), (b) flow microfluorometric determination of cell DNA content using hypotonic citrate/propidium iodide stained cells in a Cytofluorograf (Biophysics Systems; Model 4801) interfaced with a multichannel pulse height analyzer and (c) processing analyzing, and plotting cell DNA content distributions (DCD) by computer.

During the current year, 12 non-parenteral lots of methotrexate (NSC-740) containing various amounts and types of contaminants were assayed for the reversibility of toxic effects by the presence of leucovorin (Ca^{++}). Concentrations used were those which would emulate current "High Dose MTX" clinical protocols. Single lots of the 3'5'-dichloro analog (NSC-29630) and tetrahydrohomofolic (NSC-89473) were also assayed with this sytem.

Eighteen additional drugs were evaluated for their effects on L1210 cell cycle progression: dibromomannitol (NSC-94100), dibromogalactitol (NSC-104800), prospidine (NSC-166100), dianhydrogalactitol (NSC-132313), indicine-N-oxide (NSC-132319), gallium nitrate (NSC-15200), hexamethylmelamine (NSC-13875), neocarzinostatin (NSC-157365), anguidine (NSC-141537), bruceantin (NSC-165563), pyrazolo imidazole (NSC-51143), PCNU (NSC-95466), dihydro-5-azacytidine (NSC-264800), nogalamycin (NSC-70845), 7-0-CH_3-nogarol (NSC-293863), 7-0-CH_3-nogalarol (NSC-293862) and nogamycin (NSC-268450).

Preliminary studies were made to assess the feasibility of evaluating chemotherapeutic effects of antineoplastic agents on solid tumors in mice using DNA content distribution (DCD). The tumors investigated were (a) B16 melanoma, (b) colon 26, and (c) colon 38. This necessitated evaluation of a homogenization/detergent lysis technique rather than the hypotonic lysis technique used with cell culture in order to potentiate enzyme denaturation and release of parenchymal cells. In addition to tumors, normal organ tissues such as spleen, heart, liver, and thymus were examined. Concomitantly with the latter studies, several attempts were made at doing sequential biopsies on individual, anesthetized mice with AMSA (NSC-249992) treated B16 melanoma. The ultimate goal of these studies was to develop the techniques to apply to clinical therapy studies of head and neck solid tumors.

We acquired a Tektronix 4051 minicomputer which was interfaced with X-Y plotter and pulse height analyzer and have developed the necessary software for the real time acquisition and magnetic tape storage of DCD data. The software for processing and plotting the DCD profiles was also written, resulting in virtual complete independence of the DEC-10 main frame computer. Software for doing linear regression analysis was also developed.

Major Findings:

The toxic effects of Methotrexate (85-90% purity) up to 1.3 x 10^{-4}M could be 90% reversed by 1.5 x 10^{-3}M leucovorin; at 1.1 x 10^{-5}M MTX in the presence of 1.5 x 10^{-3}M leucovorin (10% growth rate inhibition of L1210) there were striking kinetic changes in DCD curves. There appeared to be little or no non-CF reversing material. This study was reported at the American Association for Cancer Research Meeting, April 5-8, 1978 in Washington, D. C. Dichloromethotrexate had 3 times the potency of MTX on a molar basis in the presence of leucovorin. Tetrahydrohomofolate was not reversed by leucovorin or L-methionine at the ID_{50} dosage.

NSC	Name	ID_{50} (μg/ml)	Primary DCD Effects
94100	Dibromomannitol	152	Late S;G2 block @ 24 hrs.
104800	Dibromogalactitol	43	" "
166100	Prospidine	605	N.A.*
132313	Dianhydrogalactitol	2.4	Late S;G2 block @ 24 hrs.
132319	Indicine-N-oxide	160	N.A.*
15200	Gallium Nitrate	120	N.A.* †
13875	Hexamethylmelamine	80	N.A.*
157365	Neocarzinostatin	0.04	G2 arrest
141537	Anguidine	0.0095	G1/S block
165563	Bruceantin	0.014	" "
51143	Pyrazolo-imidazole	115	Slow traverse thru S
95446	PCNU	3.8	Slow traverse thru S; G2 block

* N.A. DCD not run because of low potencies
† S.R.I. reports an ID_{50} of 16 μg/ml

Nogalamycin and its three analogs were analyzed as part of a collaborative study with Dr. Bijoy Bhuyan of the Upjohn Company. L1210 cells were exposed to ID_{25}, ID_{50}, and ID_{90} dosages of the four compounds and aliquots for analysis for 2, 8, and 24 hours. Effects on cell cycle distribution were time/dose dependent. Nogalamycin (NSC-70845) showed no striking changes over 24 hour period. 7-O-CH_3-Nogarol (7-O-MEN) appeared to retard transit through G2. 7-O-CH_3-Nogalorol appears to inhibit transit through S-phase with degradation of DNA at the high dose after 24 hours.

Nogamycin showed only slight changes at the lowest dose. The ID and ID_{50} dosages at 24 hours showed the beginning and extensive DNA degradation respectively.

Mouse liver (BDF/control) DCD curves were bimodal with a channel ratio of 2 and peak height ratios (G1/G2) varying from 2 to 4. Heart, lung, thymus, kidney, and spleen gave primarily unimodal peaks. Long term effects of AMSA on B16 melanoma (primarily unimodal DCD) were unsuccessful because dosage data used were dervied from saline suspension of drug injected rather than a 50% DMSO/H_2O solution. Presumably, this resulted in a higher absorption rate with must greater toxicity. Injected mice died on days 1 to 4.

Untreated colon 26 gives a bimodal DCD curve with G1 and G2 peak height ratios carrying from 1 to 4 with very few cells in S-phase.

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